UJPAH	Vol. II	No. 17	December 2014

Abstracted & Indexed by CAS, a division of American Chemical Society

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Editorial

India's emergence as major global player in Pharma sector is quite possible, If we just concentrate to produce herbal Pharma patented products of oncology which will give return in millions of dollars, though US is targeting India's patent law but hopefully we will not be harmed because there are complaints of countries as diverse as Brazil and Canada and the guidelines used by South American Nations are a carbon copy of India's section 3D. Anyhow, well researched patented herbal products of different ailments will change national economy as well as lead to more employment opportunities especially for millions of rural poor. But this would not happen unless the country enjoys major share in the international markets.

India's export in this sector have not shown much buoyancy and have not exceeded more than † 1000 Crore, whereas according to Task Force Planning Commission report (2000), the export of medicinal plants should have touched † 10,000 Crore plus by 2010. At present India's share is only about 13% of the global market while china's is just the double.

India ranks among the so called 12 mega diversity rich areas of the world and is considered one of the richest centres for plant genetic resources. Across the various ecosystems from Himalaya to Kanyakumari and from Rajasthan to North-East, about 6560 spices are estimated to be used for human and veterinary health care. Forests which contribute 90% of medicinal plants to industry are to-day under great pressure due to increasing demand and unsustainable excessive harvesting resulting into depletion of plants-diversity. Also, the analysis of various problem-areas clearly bring out that there are many other weakness and gaps in the management of the sector adversely impacting on sustainability of the sector.

To bring India top an enviable position in the list of international market, it is desired that a meaningful plan be drawn as a multi-stakeholder endeavour with a multipronged approach which looks for benefiting people, keeping environment intact and growing national economy through sustainable Development of Medicinal plant sectors.

My thanks and good wishes to all the members of the Editorial Board, Advisory Board and researchers for their valuable contributions to the release of this journal.

(Dr. S. Farooq)
Chief Editor

Antimicrobial Efficacy and Interaction of Plant Extracts With and Without Antibiotics Against Drug Resistant Bacteria

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Abstract- New sources of antimicrobial drugs need to be identified and improved strategy should be developed to combat multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating antimicrobial action needs to be exploited for their synergistic action between extracts and with antibiotics to exploit it in modern phytomedicine and combinational therapy. In the present study, alcoholic extracts of fifteen medicinal plants were screened for their antimicrobial efficacy against a wide variety of drug resistant bacteria and yeast. The extracts of *Carum copticum, C. juncea, H. spicatum, Z. officinale, S. aromaticum, Camellia sinensis, T. foenum grae*cum, *Piper cubeba, C. longa* and *A. barbadensis* showed promising action against one or more drug resistant bacteria as well as against *Candida albicans* with MIC ranged from 0.5 mg/ml to 9.5 mg/ml. Many combinations of these extracts showed synergistic action. The extract of *Carum copticum* exhibited synergy with antibiotics, tetracycline, chloramphenicol, ampicillin and gentamicin against methicillin resistant *S. aureus* which has indicated their potential to be exploited in combination drug therapy after careful evaluation *in vivo* model.

Key words: Antimicrobial activity, MDR bacteria, MIC, synergistic activity antibiotics

Introduction

The use of herbal and other natural substances is part of the fabric of traditional medicine in different part of the world. Medicinal plants have been found good source of therapeutic and novel compounds. Targeted screening of a large diversity of medicinal plants is expected to yield novel biological activities including problematic group of multidrug resistant bacterial pathogens (Ahmad et al., 2008).

Bacteria have evolved numerous defenses against antimicrobial agents and drug resistant pathogens are on the rise and such bacteria have become a global health problem. Nearly twenty years ago over 90% *S. aureus* strains were reported b-lactamase positive. Strains of b-lacatam resistant *Staphylococcus aureus* including MRSA now pose a serious problem to hospitalized patients and their care providers (Liu, et al., 2000). The production of b-lactamase is recognized as one of the main mechanism of bacterial-resistance to b-lactamase antibiotics. Numerous compound have been included in the list of b-lactamase inhibitors and some of these have shown potential clinical usefulness based on their synergistic-effects when they are combined with b-lactamase-labile antibiotics. Many b-lactamase were found to be resistant to b-lactamase inhibitors. Similarly multidrug resistant problem is common in members of family Enterobacteriaceae specially *E.coli*, *Salmonella*, *Shigella* and several other humans and animal pathogen like *Haemophilus influenza*, *Campylobacter*, *Pseudomonas* aeruginosa, *Mycobacterium tuberculosis* both in developing and developed countries (Eldelstein *et al.,* 2001; Tonkic *et al.,* 2005; Ahmad et al., 2008).

India has one of the world's richest flora with about 120 families of plant comprising 1, 30,000 species. A large portion of the world population especially in the developing countries depends on the traditional-system of medicine for a variety of diseases. The world health organization (WHO) reported that 80% of the world's population rely chiefly on traditional medicines and major part of the traditional therapies in-

volve the use of plant extracts or their active constituents (WHO 1993).

According to an estimate about 119 secondary plant metabolites are used globally as drugs. It has been estimated that 14-28% of higher plant species are used medicinally, that only 15% of all angiosperms have been investigated chemically and that 74% of pharmacologically active plant derived components were discovered after following upon ethanobotanical use of plants (Eloff, 1998). The plants are valuable in the three basic ways: (1) they are used as source of direct therapeutic agent. (2) As a source of new bioactive metabolites including antimicrobial, antihelminthic and antiprotozoan etc. (3) they serve as raw material base for elaboration of more complex semisynthetic chemical compounds.

According to a report published in the 'Journal of the American Medical Association', more than 630 million visits are made to alternative practitioners each year in U.S. also more than 15 million adults take herbal remedies while taking other medication (Hoffman, 2004).

Concerted efforts have been made all over the world to explore the various biological and specific pharmacological activities and their active compounds all over the world. The antibacterial and antifungal activities of Indian medicinal plants are widely known against a variety of pathogenic and opportunistic microorganisms (Aqil and Ahmad, 2007). However, targeted screening with improved strategy to evaluate the efficacy of various potential plants against problematic multi drug resistant bacteria is in the stage of infancy. It is expected that plant extract showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of plant extract (Lee *et al.,* 1998). In the recent years plants have been screened against multidrug resistant bacteria including *Staphylococcus aureus*, *Salmonella paratyphi, Escherichia coli, Shigella dysentriae* and *Candida albicans*. The selection of medicinal plant was based on their traditional uses in India and reported antimicrobial activity of many medicinal plants (Chopra *et al.,* 1992; Ahmad *et al.,* 1998; Mehmood *et al.,* 1999). The recent development in the phytopharmacology is development of multicombinational drug against multidrug resistant bacteria. This has been possible due to interaction among plant extracts (Phytocompounds) and with other chemotherapeutic agents that may be synergistic or additive in their interaction. The development of these drugs has grown a new future in the area of phytopharmacology and medical practices.

At present multi-drug therapy or combinational antibiotic therapy is in use. However its efficacy may be severely hindered against several MDR bacteria. Therefore, there is an increased request to develop novel drugs against multi drug resistant bacteria. One possible approach is to screen/unexplored Indian medicinal bioactive plant extracts for their potential to be used against multi drug resistant bacteria.

Considering the vast potential of Indian medicinal plants as an anti-infective agent, we have selected 15 plants on the basis of their traditional uses, ethanopharmocoloical data and local availability. The present screening programme has been planned to identify most effective plants with broad spectrum activity against drug resistant microbial pathogens and to assess synergy with antibiotics *in vitro.*

Material and Methods

Plants material

The authentic plant material was obtained from the Himalaya Drug Company, Dehradun and some are collected directly in the vicinity of Aligarh University campus. The identification of the samples was further confirmed by the plant taxonomist in the Department of Botany, Aligarh Muslim University, Aligarh. The voucher specimen has been deposited in the Department of Agricultural Microbiology, Faculty of Agricultural Sciences, AMU, Aligarh as shown in Table- 2.

Drug resistant and sensitive bacterial strains used in the screening programme

The Standard strains were obtained from different National and International Culture Collection Centers/ Collection of individual scientist and clinical isolates were collected from Department of Microbiology, J N Medical College, AMU, Aligarh. Multidrug resistant bacteria include the strains of *Shigella, Salmonella typhi*, *Staphylococci* including methicillin resistant *Staphylococcus aureus* (MRSA), *Psuedomonas aeruginosa* and R-plasmid harbouring strains of *E. coli.* MRSA and

some other Gram positive and Gram negative bacteria were also used in our laboratory. The details of the test strains and their relevant characteristics are mentioned in Table- 1.

Chemicals and Antibiotics

All the antibiotic discs were purchased from Hi-Media Lab Pvt Ltd, Mumbai, India. The indicator dye p-iodonitro tetrazolium violet were purchased from Sigma Chemical Co., USA. MMS and Sodium azide were purchased from Sisco Reseach Laboratory, India. All the other media/chemicals used were of analytical grade.

Bacterial cultures

Bacterial isolates were obtained from different sources (Table-1) and were subjected to antibiotic sensitivity by disc diffusion method (Bauer et al., 1966).

ß-lactamase production

The method described earlier (Ahmad et al., 2008) was used for detection of proudtcion of ß-lactamase.

Culture Media and Inoculum preparation

Nutrient broth/ Agar and Muller–Hinton broth/ agar (Hi-Media Pvt. Ltd., Mumbai, India) were used to grow the test bacteria at appropriate temperature 30-37 °C for 18hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^5 –10^6 CFU/ml.

Preparation of plant extracts and its fractionation

Plant extract was prepared as described earlier (Ahmad and Beg 2001) with a little modification. 800 gram of dry plant powder was soaked in 2.5 liter of 70% ethanol for 8–10 days and stirred after every 10 hrs using a sterilised glass rod. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). This alcoholic filtrate was concentrated under vacuum on rotary evaporator at 40 °C and then stored at 4 °C for further use. The crude extract was prepared by dissolving known amount of the dry extract in DMSO, to have a stock solution of 100 mg/ml concentration.

Antimicrobial assay

The agar well diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Beg 2001) was used. 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100µl of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol) at 100µg/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.

Minimum inhibitory concentration of plant extracts

Minimum inhibitory concentration of plant extracts against test bacterial strains was determined by tube broth dilution method, using specific dye (p-iodonitro tetrazolium violet) as an indicator of growth (Eloff 1998). 2 ml of the plant extract was mixed with 2 ml of Muller-Hinton broth (Hi-Media Ltd., Mumbai, India) and serially diluted into the next tube and so on. 2 ml of an actively growing culture of different test strains was added before incubating for over night, at 37 °C. After examining turbidity visually, 0.8 ml of 0.02 mg/ml indicator dye (p-iodonitro tetrazolium violet) was added to each tube and incubated at 37 °C. The tubes were examined for the colour development after 30 min. Absence of growth was also confirmed by spreading 0.1 ml of broth from such test tube on normal nutrient agar plate.

Synergistic interaction of plant extracts with antibiotics

Synergistic interaction between antibiotics like ampicillin, tetracycline and chloramphenicol with crude plant extracts was studied by agar well diffusion method. For determining the synergistic effects of plant extract with antibiotic, the wells were punched at a predetermined distances so that their inhibitory circles touch each other only tangentially without influencing each other as recommended by Ahmad et al. (2008). The wells were inoculated with plant extract and antibiotic separately. Plates were then incubated at 37 °C, for 18 hrs. Enlargement of inhibition zones indicates a positive interaction (synergism).

Phytochemical analysis of plant extracts

Major phytocompounds in the crude extracts of plants, were detected by standard colour tests and thin layer chromatography, as described elsewhere (Ahmad and Beg 2001).

Results and Discussion

Antimicrobial activity of plant extracts against drug resistance pathogenic bacteria

Multiple drug resistance in pathogenic bacteria has emerged as important problem in many countries of the world. There are now increasing case reports documenting the development of clinical resistance to newer and broad spectrum antibacterial drugs like fluroquinolone (norfloxacin, ciprofloxacin, oflaxacin etc.) in many pathogenic bacteria. In the present study, clinical isolates of *S. aureus P. aeruginosa, Shigella spp. E. coli, Citrobacter spp., B. subtilis* and *Candida albicans* were used. These microbial strains are found to be resistant to one or more antibiotics, showing the common occurrence of drug resistance. These finding are in agreement with the reports of previous workers as these strains have been previously tested for their sensitivity to antibiotics (Ahmad and Arina, 2001; Aqil et al., 2005, Aqil and Ahmad, 2007; Jafri et al., 2014). Further, these test isolates of bacteria were also tested for the production of b-lactamases (Table- 1).

In the present study, 15 medicinal plants were selected on the basis of their traditional uses in treatment of different disease in India and worldwide. Only alcoholic extracts of plant material have been used as the alcohol was found suitable solvent for the extraction of antimicrobially active constituents from plants (Eloff,1998;Ahmad et al.,1998).

The details of collected plant materials, their ethanobotanical data and parts used have been given in the Table- 2. Antibacterial activity of crude extracts of the 15 medicinal plants against Gram positive bacteria (7 distinct isolates of *S. aureus* and *B. subtilis*) and Gram- negative bacteria (*E.coli*, *P. aeruginosa, Citrobacter* and *Shigella spp.*) and a yeast (*C. albicans*) is presented in Table- 3 and 4. Activity of ethanolic curde extracts against Gram positive bacteria showed broad spectrum by *A. barbadensis*, *C. copticum*, *C. juncea, C. sinensis, H. spicatum, P. guajava, S. aromaticum* and *Z. officinale* (Table- 3). On the other hand broad spectrum activity against Gram negative MDR bacteria was exhibited by only *C. copticum*, *C. spicatum, C. juncea, S. aromaticum* and *T. foenum graecumas* as evidenced from their activity against more than 3 test bacteria with fair size of zone of inhibition (Table- 4). Most potential plant extract was *S. aromaticum, C. copticum* and *C. juncea*. Other plant extracts have also demonstrated strong antibacterial activity against one or more bacteria. Our findings are correlated with reports of earlier workers on *S. aromaticum*, *T. foenum graecum, P. cubeba, C. longa* & *C. sinesnsis* (Ahmad et al., 1999; Lacobellis et al., 2005). While activity of *C. copticum, C. juncea, H. spicatum, A. barbadensis* against MDR bacteria are probably reported for the first time. Similarly, anticandidal activity of these 15 plant extracts demonstrated that 10 plants could exhibit varying level of activity (Table- 4). Highest activity in terms of radius of zone of inhibition was recorded in *C. copticum* followed by *C. juncea and S. aromaticum*. Five plants could not show any anticandidal activity. Similar findings have been reported by Mehamood et al., 1999 and few other workers (Aqil and Ahmad, 2007). Over all sensitivity of MDR bacteria against plant extracts showed that *P. aeruginosa* strain is more sensitive followed by *S. aureus, P. aeruginosa, B. subtilis, Shigella spp.* and *E. coli*.

On the basis of broad spectrum activity of plant extracts four less commonly studied plant extracts were evaluated for their potency in terms of minimum inhibitory concentration against a variety of MDR bacteria as shown in Table- 5. MIC values of *C. copticum* varied greatly from 0.51 mg/ml to 4.62 mg/ml against test bacteria. Similarly, MIC ranged from 2.26 mg/ml to 9.49 mg/ml (*C. juncea*), 1.70 to 6.25 mg/ml (*H. spictaum*) and 1.23 to 6.60 mg/ml (*Z. officinale*). Variation in MIC values might be due to difference in cell well composition and intrinsic tolerance of the test isolates, nature and composition of phytoconstituents. Phytochemcial analysis of active plant extracts of six plants was made for the presence of major phytocompounds like alkaloids, flavonoides, glycoside, phenols & tannins as depicted in Table- 6. The presence of one or more major phytoconstituents was detected in the crude extracts by colour test and or by TLC (Fig.1).The differences in their phytocompounds might be responsible for varied activity & MIC values. These observations were supported by many workers (Nakamura et al., 2002; Shanab et al., 2004). Thus our antimicrobial screening results also justify the traditional uses of these plants in ailments and localized skin infections caused by *S.aureus, E.coli, Shigella spp., P.aeruginosa,* and *Candida albicans*.

Synergism in plant extracts

In the traditional systems of medicine (Ayurveda and Unani-Tibbiya) formulation of herbal drugs are prepared as a mixture of many crude extracts in different preparations. It is commonly believed that various active phytoconstituents of plant extracts posses additive or synergistic activity. Therefore, 14 plant extracts were selected on the basis of their antimicrobial activity against *S. aureus* and were tested in different combinations by agar well diffusion method (Table- 7 and 8). Significant activity was detected in different combination such as (1) *C. sinesis and H.spicatum* (2) *C. sinensis and P. guajava* (3) *C. sinensis and C. longa* (4) *C. copticum and S. aromaticum* (5) *C. copticum and M. arvensis* (6) C. *copticum and Z. officinale* (7) *H. spicatum and P. guajava* (8) *C. copticum and C. sinensis* (9) *P .zeylanica and H. indicus* (10) *C. copticum and C. juncea*. The synergism in some of the above interaction is shown in plate- 1 and 2.

This preliminary investigation suggested that it would be wise to evaluate the possible additive, synergistic or antagonistic interaction of crude plant extracts in different combinations to obtain enhanced activity of herbal preparations, although, it will also require an additional data on *in vivo* studies.

Multiple antibiotic therapy is now considered an effective way to control infectious diseases caused by drug resistant bacteria. Phytocompounds which may have strong activity against antibiotic resistant bacteria is expected to give strong synergistic and additive effect with antibiotics. Considering this known fact, we have tried to see the possible synergistic effect between plant extracts *C. copticum* and antibiotics. *C. copticum* extract showed synergistic interaction with tetracycline, chloramphenicol, ampicillin and gentamycin against mutlidrug resistant *S. aureus* (MRSA) strain. The above findings show that synergistic interactions are specific and the possible reason may be found in the interaction of different phytoconstituents with antibiotics. This result agrees with the observation of synergistic interactions of medicinal plants with chloramphenicol as reported by Lee (1998) and Aqil et al., (2005).

Conclusion

This preliminary investigation indicated that potential plant extracts showing broad spectrum antimicrobial activity and synergy could be further tested to determine the efficacy *in vivo* against MDR bacteria. Active fractions of various plants may also be exploited in preparation of herbal formulation of improved efficacy and quality.

References

1. Ahmad, I. and Beg, A.Z. Antimicrobial and Phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. *J. of Ethanopharma*., 2001; 74: 113-123.
2. Ahmad, I.; Mehmood, Z. and Mohammad, F. Screening of same Indian medicinal plants for their antimicrobial properties. *J. of Ethanopharma*., 1998; 62: 183-193.
3. Ahmad, I.; Zahin, M.; Aqil, F.; Hasan, S.; Khan, M.S.A. and Owais, M. Bioactive compounds from *Punica granatum, Curcuma longa,* and *Zingiber Officinale* and their therapeutic potential (Review). *Drug of the Future,* 2008; 33(4): 01-18.
4. Ahmad, I.; Zaiba; Beg, A.Z. and Mehmood, Z. Antimicrobial potency of selected medicinal plants with special interest in activity against phytopathogenic fungi. *Ind. Veter. Med. J.,* 1999; 23 (10): 01-08.
5. Aqil, F. and Iqbal Ahmad Antibacterial properties of traditionally used Indian medicinal plants. *Methods & Finding Experim. Clin. Pharmacol.,* 2007; 29(2): 79-92.
6. Aqil, F.; Khan, M.S.A.; Owais, M. and Iqbal Ahamd Effect of certain bioactive plant extracts on clinical isolates of â-lactamase producing methicillin resistant *Staphylococcus aureus*. *J. Basic Microbiol.,* 2005; 45: 106-114.
7. Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C. and Turch, M. Antibiotic susceptibility testing by standardized single disc method. *AM. J. Clini. pathol*., 1966; 45: 493-496.
8. Chopra, R.N.; Nayer, S.L. and Chopra, I.C. (1992)

Glossary of Indian medicinal plants (3rd Edn.) Council of Scientific and industrial research New-Delhi (India): 246-7.

9. Edelstein, M.; Pimkin, M.; Edelstein, I.; Drithachenko, T.; Semenov, V.; Stratchounslei, L. (2001). Clonal spread of Cefotaxime-Resistant. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy.
10. Eloff, J.N. Which extract should be used for the screening and isolation of antimicrobial components from plants. *J. of Ethanopharm*, 1998; 60: 1-8.
11. Hoffman, R. (2004). Article on the power of plants. drhoffman.com.
12. Jafri,H.; Husain, F.M.; Ahmad, I. Antibacterial and antibiofilm activity of some essential oils and compounds against clinical strains of *Staphylococcus aureus*. *J.biomed.ther.sci.*, 2014; 1(1):65-71.
13. Lacobellis, N.S.; Cantore, P. Lo.; Capasso, F. and Senatore, F. Antibacterial activity of *Cuminum cyminun* L. and *Carum carvi* L. essential oils. *J. Agric, Food, Chem.*, 2005; 53 (1): 57-61.
14. Lee, C.K.; Kin, H.; Moon, K.H. and Shun, K.H. Screening and isolation of antibiotic resistance inhibitors from herb material resistance inhibitors inhibition of volatile components of Korean aromatic herbs. *Arch. Pharma. Res.*, 1998; 21 (1): 62-66.
15. Liu, X.I.; Durham, D.G. and Richards, R.M.E. Baicalin synergy with b-lactam antibiotics against methicillin resistant *staphylococcus aureus* and other b-lactam-resistant strains of *S. aureus. J. Pharm. Pharmacol.*, 2000; 52: 361.
16. Mehmood, Z.; Ahmad, I.; Mohammad, F. and Ahmad, S. Indian Medicinal Plants: A potential source for anticandidal drugs. *Pharm. Biol.*, 1999; 37 (3): 237-242.
17. Nakamura, C.V.; Fillo, B.P.D.; Cortez, A.G.C.; Sanches, N.R.; Persine, G.L.; Holetz, F.B. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases Mem. *Inst. Oswaldo Cruz. Rio de janeiro.*, 2002; 97(7): 1027-1031.
18. Perez, C.; Pauli, M. and Bazerque, P. An antibiotic assay by well diffusion method. *Acta. Bioloziae et Med. Exper.*, 1990; 15: 113-115.
19. Shanab, A.B.; Adwan, G; Safiya, A.D.; Adwan, K.; Jarrar, N. Antibacterial activities of some plant extracts utilized in popular medicines in Palestine. *Turk. J. Biol.,* 2004; 99-102.
20. Tonkic, M., Goic-Barisic, I.; Punda-Polic, V. Prevalence and antimicrobial resistance of extended spectrum of extended spectrum of b-lactamases producing *E. coli* and *K. pneumoniae* strains isolated in a university hospital, split, croatia. *Int. Microbial,* 2005; 8 (2): 119-24.
21. World Health Organization. Summary of WHO guide lines for the assessments of herbal medicines. *Herbal Gram,* 1993; 28: 13-14.

Table-1 Antibiotics resistant pattern and β-lactamase production by test strains

Name of bacteria	Strains code	β-lactamase hydrolyzing β-lactam antibiotics		Resistant pattern of used strains against antibiotics
		Ampicillin	Benzyl penicillin	
Staphylococcus aureus	SA-03	+	+	Cx, M, A. Pn, Cf, Do, Sm, Na
Staphylococcus aureus	SA-08	–	–	Cx, M, A, Pn, Cf, Sm,
Staphylococcus aureus	SA-11	+	+	Pn, Am, M, S, T, Do, Na, Cu,
Staphylococcus aureus	SA-21	+	+	Cx, M, A, Pn, Cf, Do, Sm,
Staphylococcus aureus	SA-22	+	+	Sensitive to all drugs
Staphylococcus aureus	SA-28	+	+	Pn, Am, Cx, Cf, M, Pc, Kt, T, S,
Staphylococcus aureus	SA-29	+	+	Cx, M, A, P,
E.coli	UP-2556	–	–	Pn, A, Cx, Do,
E.coli	EC-14	+	+	Pn, A, Cx, M, Ce, Cfx, Cep, Cu,
E.coli	EC-20	+	+	Pn, A, Cx, M, Ce, Cfx, Cu, Va, T, E,
Citrobacter sp	SM-06	+	+	Pn, A, Cx, M, Co, T, C, Do, Nx, Nf, Na, Cu
Shigellasp.	SM-07	+	+	Pn, Cx, M, Co, Cf, T Do C, Na
Shigellasp.	SM-08	+	+	Pn, A, Cx, Co, Ce, Cf, Cfxx, Na
Citrobacter sp	EN-06	+	+	Pn, Cx, M, T, C, Do, Nx, Na, E,
P. aeruginosa	*P.aeruginosa*	NT	NT	A, C, T, Na, Co, Cx, Am, M
B. subtilis	*BS*	–	–	Sensitive

Pn, Penicillin, A, Ampicillin; Cx, Cloxacillin, Ce, Cephotaxime; Cu, Cefuroxime; Cfx, Cefixime, Cefpodoxime; M, Methicillin; Va, Vancomycin; Nf, Nitrofurantoin, Nx, Norfloxacin, Nv, Novobiocin Co, Co-trimoxazole; Na, Nalidixic acid; T, Tetracyline; C, Chloramphenicol; Do, Doxycycline; E, Erthromycin.

Table 2 Ethanobotanical data and traditional uses of medicinal plants.

S. No.	Scientific Name (Family) V-Sp.-No.	Vernacular name	Part Used	Site of Collection	Known Phytocompounds	Traditional Uses
1	*Aloe barbadensis* Mill (Liliaceae) IOA-66/05	Ghee kavar	Leaves	Cultivated in R.A.K. Institute AMU Aligarh	Aloin , Iso-barbaloin, Emodin, Resin, Anthra quinone, Oxidase, Catalase, Lignan, Salicylic acid, Saponins, Sterols and Triterpenoids (Wendell and Combert; 2004)	Used in wound healing, treatment of burnt skin, Protection of skin from radiation and laxative (Wendell and Combert, 2004).
2	*Bombax cieba* L (Bombaceae) IOA-64/05	Semal	Flowers	Locally purchased	lup-20 (29) en-3b-ol, 2-hexyl-7, 8-dimethyl-1, 4-naphthaquinone (Ansari, 2004)	Used as Aphrodisiac, Laxative, anti- hemorrhagic and diarrhea (Oudhia, 2003).
3	*Carum copticum* Benth. & Hook. (Umbelliferae) IOA-62/05	Ajwain	Seeds	-do –	Essential oil, thymol (Chopra *et al.*, 1992).	Used in stomach, curmin, antiseptic, tonic, diarrhea, flatulence and cholera. (Chopra *et al.*, 1992)
4	*Camellia sinesis L.* IOA-62/05	Tea	leaves	-do-	Caffeine, tannins, catechin, theflavin (khare, 2007)	Stimulant, diuretic, astringent (khare, 2007)
5	*Crotalaria juncea L.* / Fabiaceae / IOA-63/05	Sana	Leaves	-do-	Juneceine, senecionins, seneciphylline (Morris, 1999)	Used in purification of blood, in impetigo, Psoriasis, Poisnous to livestock (Chopra *et al*, 1992). Also used as Antitumor hypotensive (Morris, 1999).
6	*Curcuma longa* L. (Zingiberaceae) IOA- 68/05	Haldi	Rhizomes	-do-	Curcumin, Alkaloids, Zingiberine, Ketone, Alcohol (Chopra *et al.*, 1992).	Used in Indian cuisine, mustard and curry powder providing colour and flavour. It is used as antitumor, anti-inflammatory, antioxidant, and anti-infectious activities (Chopra *et al*, 1992).
7	*Hedychium spicatum* Ham. ex smith. (Zingiberaceae) IOA-50/05	Kachri	Fruits	-do-	Essential oils, methyl-paracumarin, acetate, cinnamic ethyl-acetate (Harborne and Baxter, 1995).	Good in liver complaints, vomiting, diarrhea, inflammation and pain in snake bites, as tonic stomachic (Harborne and Baxter, 1995).
8	*Mentha arvensis* L (Labiatae) IOA-67/05	Peppermint	Leaves	CIMAP, Lucknow.	E.oil-d-carvone, carene,de-Sylvestrene & citronellol (Chopra *et al.*, 1992).	Used in fragrance, in get rid from bed breath, treatment of tonsillitis, Coryza and cough Oudhia, (2001).
9	*Piper cubeba* L. (Pipperaceae) IOA-65/05	Kabab chini	Seeds	Locally purchased	E.oils, cubebin (Chopra *et al.*, 1992).	Used in genito-urinary diseases like cystitis, gonorrhea (Chopra *et al.*, 1992).
10	*Piper nigrum* L (Piperaceae) HDCO-85/05	Gol mirch	Seeds	-do-	Alkaloids, Piperin, piperidine, E. oils (Chopra *et al*, 1992)	Used in weakness following fever, vertigo, coma, as stomach, in flatulence and in arthritic diseases (Chopra *et al.*, 1992).
11	*Psidium guajava* L (Rosaceae) IOA-23/05	Guava	Leaves	AMU campus Aligarh.	Astrin, Euginol (Chopra *et al.*, 1992) Flavonoids and Tannis (Nakamura *et al.*, 2002).	Used in ulcers, wounds cholera & diarrhea (Chopra *et al.*, 1992). Leukorrhia, and skin diseases (Nakamura *et al.*, 2002).
12	*Raphanus sativus L.* / Brassicaceae/ IOA-24/05	Muli	Leaves	-do-	Glucaldehyde, Enzymes and methylmercaptane (Chopra *et al*, 1992).	Used for urinary complaints, piles and gastrodynic pains. (Chopra *et al*, 1992).
13	*Syzygium aromaticum* L. (Myrtaceae) IOA-26/05	Clove oil	Buds	CIMAP, Lucknow.	E.oil-Euginol (Analyst; 1909). Vanilline (Harborne and Baxter, 1995).	Used in baked goods, sauces & as antidiabitic agents. (Chopra *et al.*, 1992).

14	*Trigonella foenum graecum* L. (Leguminosae) IOA-61/05	Methi	Seeds	Locally purchased	Alkaloids Trigonellin and cholline, E.oils, Saponins, Nicotinic acid (Chopra *et al*, 1992). Galactomannan and saponin (Ammar *et al.*, 1999).	Used in small pox, dysentry and for cooling (Chopra *et al.*, 1992). Ant diabetic and hypocholestrolemic (Ammar *et al.*, 1999).
15	*Zingiber officinale* Rosc. (Zingiberaceae) HDCO-01/05	Ginger	Rhizomes	-do-	Potassium-oxalate, camphene, β phellandrene, Zingiberene, cineol, citral, Borneol, E.oil (Chopra *et al.*, 1992).	Traditionally used in Asian and Indian dishes. Exhibited good antioxidant activity (Chopra *et al.*, 1992), cough, cold, fever, muscles aches and nausea. Also used as contraceptive and fertility agent (Ficker *et al.*, 2003).

Table-3 Antibacterial activity of plant extracts against Gram positive bacteria

S.No.	Scientific Name (Family)	Percent Yield	Antimicrobial activity (Radius in mm) ± SD							
			SA-03	SA-08	SA-11	SA-21	SA-22	SA-28	SA-29	MTCC 121*
1.	*Aloe barbadensis*	1.20	8.50± 0.50	6.33 ± 0.28	11.06± 0.1	6.33± 0.28	–	11.23±0.25	6.33±0.28	–
2.	*Bombex cieba*	5.40	–	–	–	–	–	6.30±0.26	–	–
3.	*Carum copticum*	6.25	–	12.2 ± 0.25	9.33± 0.41	8.5±0.24	18.2± 0.2	10.16±0.28	11.33±0.28	17.70±0.65
4.	*Crotalaria juncea*	4.00	7.40 ± 0.55	9.40 ± 0.17	8.13± 0.25	–	6.30±0.26	8.46± 0.41	8.23±0.25	9.20±0.15
5.	*Curcuma longa*	4.20	–	–	7.06± 0.11	–	–	–	6.23±0.25	8.16±0.50
6.	*Camellia sinesis*	10.20	9.20±0.12	8.23 ± 0.25	10.23±0.25	–	8.33±0.28	6.33 ± 0.28	8.33±0.28	–
7.	*Hedychium spicatum*	3.60	9.20± 0.12	6.23 ± 0.25	11.33±0.28	–	–	6.23 ± 0.25	7.4±0.58	6.26±0.25
8.	*Mentha arvensis*		7.06± 0.11	–	–	5.23±0.25	–	7.13±0.11	8.23±0.25	–
9.	*Piper cubeba*	7.17	–	–	7.16± 0.28	–	–	7.26± 0.25	–	8.23±025
10.	*P. guajava*	5.25	8.66± 0.50	8.33± 0.28	6.66± 0.28	–	–	–	6.33±0.28	–
11.	*Piper nigrum*	5.89	–	–	–	–	7.26±0.25	–	–	–
12.	*Racinus sativus*	5.25	–	8.33 ± 0.28	–	–	–	–	–	–
13.	*Sysygium aromaticum*		8.64± 0.55	7.33± 0.41	8.23± 0.25	7.26±0.25	–	11.23±0.25	7.26± 0.25	–
14.	*Trigonella foenum graecum*	2.94	–	8.23± 0.41	–	–	–	–	7.33±0.41	–
15.	*Zingiber officianle*	4.32	–	8.23 ± 0.28	9.16±0.28	–	9.13±0.11	6.26±0.25	–	–

* MTCC 121, *Bacillus subtilis,* SD – Standard deviation

Table-4 Antibacterial activity of plant extracts against Gram negative bacteria

S. No.	Scientific Name (Family)	Antimicrobial activity (Zone in mm) ± SD*							
		SM-06	SM-07	SM-08	UP 2566	EC-14	EC-20	P	CA
1	*Aloe barbadensis*	–	10.1±0.2	11.2±0.2	–	6.3±0.2	–	–	–
2	*Bombex cieba*	–	–	–	–	–	–	–	–
3	*Carum copticum*	12.1± 0.6	15.2±0.4	9.2± 0.1	13.0 ±0.4	8.2± 0.2	8.2± 0.2	6.4±0.2	18.3± 0.1
4	*Crotalaria juncea*	–	9.2± 0.1	10.1±0.2	–	13.0± 0.4	–	6.4±0.2	10.1±0.2
5	*Curcuma longa*	–	–	–	–	8.2± 0.2	–	6.3± 0.2	7.1±0.1
6	*Camellia sinesis*	–	10.2±0.2	9.3±0.3	–	–	–	–	6.2±0.2
7	*Hedychium spicatum*	6.2±0.2	8.5±0.3	7.0±0.2	7.5±0.2	8.8±0.4	8.5±0.5	6.4±0.2	8.2±0.2
8	*Mentha arvensis*	5.2±0.2	–	6.3±0.2	5.2±0.2	–	10.1±0.2	–	5.2±0.2
9	*Piper cubeba*	–	6.3±0.4	–	–	8.4±0.2	7.5±0.5	6.4± 0.2	8.2±0.2
10	*P. guajava*	5.2±0.3	–	–	6.4±0.5	7.2±0.2	7.5±0.5	–	–
11	*Piper nigrum*	–	–	–	–	–	–	–	7.2±0.2
12	*Racinus sativus*	–	–	–	–	–	–	6.4±0.2	–
13	*Sysygium aromaticum*	8.2± 0.2	19.1±0.4	8.2±0.2	7.3±0.4	8.2±0.2	8.2± 0.2	5.2± 0.2	8.6±0.5
14	*Trigonella foenum graecum*	–	13.2±0.1	12.4±0.4	13.8±0.4	11.6±0.4	12.3±0.8	–	–
15	*Zingiber officianle*	–	–	–	6.2±0.3	8.2±0.2	7.5±0.2	–	8.2±0.4

*SD – Standard deviation

- = No activity.

Table-No. 5 Activity profile of crude plant extracts in terms of Minimum inhibitory concentration (MIC)

S. No.	Plant Extract	Yield in mg/100 gm of dry powder	Minimum inhibitory concentration against test microorganisms (mg/ml)															
			SA						B	C		S		EC				
			SA-03	SA-08	SA-11	SA-21	SA-28	SA-29	MTCC	EN-06	SM-06	SM-07	SM-08	EC-M	EC-14	EC-20	P	CA
1	*C. copticum*	6.25	4.5	4.62	4.62	0.51	1.38	4.16	4.16	1.38	0.51	0.51	0.51	4.16	2.08	2.08	NT	1.38
2	*C. juncea*	4.0	8.54	2.84	2.84	NT	2.84	2.84	9.49	NT	NT	4.26	2.84	NT	4.26	NT	2.84	3.70
3	*H. spicatun*	3.6	4.21	4.21	3.33	NT	3.70	6.25	6.25	3.33	3.33	NT	1.70	1.70	NT	3.33	6.25	4.26
4	*Z. officinale*	4.32	3.33	3.33	3.33	3.33	3.0	6.66	6.66	NT	NT	NT	1.70	NT	1.70	NT	NT	1.23

NT - Not tested.

Organisms key : SA – *Staphylococcus aureus*, B – *Bacillus subtilis*, C - *Citrobacter* spp., S – *Shigella* spp., EC - *E.coli*, P – *Pseudomonas aeruginosa*, CA- *Candida albicans* .

Table-6 Phytochemical analysis of active plant extracts for major bioactive compounds

S. no.	Plant name	Part used	Phytocompounds detected					
			Alkaloids	Flavonoids	Glycosides	Phenols	Tannins Epi/ gallo	Condensed salts
1.	*Aloe barbadensis*	Leaves	+	–	–	+	+	–
2.	*Carum copticum*	Seeds	+	+	+	+	+	+
3.	*Crotalaria juncea*	Leaves	–	–	–	–	–	–
4.	*Hedychicum spicatum*	Fruit	+	+	+	–	–	–
5.	*Psidium guajava*	Leaves	+	+	–	+	+	–
6	*Zingiber officinale*	Rhizomes	+	–	+	–	–	–

Table 7 Synergistic interaction among plant extract

Strains Used	Plants Extract (A)	r_A (in mm)	Plant extract (B)	r_B (in mm)	Combined radius (r_A+r_A) in mm	Enlargement of Zone-size (in mm)	Synergism
S. aureus (SA-03)	*C. sinensis* *Z. officinale* *C. longa* *C. sinensis* *C. copticum* *C. copticum*	9.2 ± 1.1 7.9 ± 1.5 6.8 ± 0.4 17.1± 0.4 10.3 ± 1.5 10.3 ± 1.5	*Z. officinale* *C. sanctum* *P. guajava* *C. longa* *S. aromaticum* *M. arvensis*	7.9 ± 1.5 6.8 ± 0.4 8.3 ± 0.2 14.0 ± 0.7 8.2 ± 1.2 7.01 ± 0.60	17.1 ± 0.8 14.7 ± 1.8 15.2 ± 0.5 35.2 ± 0.4 24.2 ± 0.4 21.4 ± 0.1	– – – 4 6 4	– –. – + + +
SA-08	*C. copticum* *C. sinensis* *H. spicatum* *H. spicatum* *C. copticum* *C. copticum* *C. copticum*	16.0 ± 0.7 8.2 ± 1.2 9.0 ± 0.4 9.0 ± 0.4 12.1 ± 0.6 10.3 ± 1.6 10.3 ± 1.6	*C. sinensis* *Z. officianle* *R. sativus* *P.guajava* *Z. officinale* *S. aromaticum* *M. arvensis*	8.2 ± 1.2 7 ± 0.5 8.4 ± 0.3 9.3 ± 0.1 7.1 ± 0.8 7.1 ± 0.9 –	27.1± 0.8 15.2 ± 0.6 17.7 ± 0.6 22.5 ± 0.6 24.3 ± 0.9 23.6 ± 1.1 17.1 ± 0.8	3 – – 4 5 6 –	+ – – + + + –
SA-11	*P. guajava* *P. guajava* *H. spicatum* *C. spicatum* *C. copticum*	10.3 ± 0.4 10.3 ± 0.4 9.0 ± 0.8 9.3 ± 0.4 9.3 ± 0.4	*C. sinensis* *H. spicatum* *C. sinensis* *S. aromaticum* *M. arvensis*	7.3 ± 1.7 9 ± 0.8 7.2 ± 1.7 8.6 ± 1.0 –	17.1 ± 0.8 23.0 ± 0.8 19.5 ± 0.4 25.1 ± 0.3 –	– 4 3 8 –	– + + + –
SA-21	*Plumbago zeylanica* *Punica granatum*	15.2 ± 0.5 8.2 ± 0.9	*H. indicus* *H. antidysenterica*	10.3 ± 1.8 10.2 ± 1.8	32.1 ± 0.8 18.3 ± 1.4	7 –	+ –
SA-22	*Plumbago zeylanica* *Punica granatum* *Plumbago zeylanica* *Hemidesmus indicus*	15.2± 0.4 12.1± 0.6 14.6 ± 1.0 14.7± 1.7	*H. indicus* *H. antidysenterica* *Acorus calamus* *H antidysenterica*	14.6± 1.9 8.2 ± 1.0 15.2± 0.2 8.3 ± 1.0	33.2 ± 0.3 20.4 ± 0.8 32.9 ± 0.4 29.0± 0.6	4 - 3 7	+ - + +
SA-28	*C. sinensis* *P. guajava* *Z. officinale* *H. spicatum* *H. spicatum* *C. copticum* *C. copticum*	6.2 ± 0.2 6.6 ± 0.2 6.73 – 5.4 ± 0.5 10.4 ± 0.3 10.4 ± 0.5	*P. guajava* *Z. officianale* *C. longa* *Z. officinale* *P. cubeba* *S. aromaticum* *M. arvensis*	6.6 ± 0.2 6.7 ± 0.3 – 7.1 ± .02 7.1 ± 0.2 11.3 ± 0.3 7.4 ± 0.3	12.6 ± 0.7 13.0 ± 0.3 – 12.1 ± 0.230 12.1 ± 0.230 – –	– – – – – – –	– – – – – – –
SA-29	*C. copticum* *C. sinensis* *C. sinensis* *Z. officinale* *C. juncea* *C. copticum* *C. copticum*	11.1 ± 0.5 14.6 ± 1.5 – 6.7± 0.5 8.3± 0.1 7.4 ± 0.4 7.4 ± 0.4	*C.juncea* *P. guajava* *Z. officinale* *C. juncea* *H. longa* *S. aromaticum* *M. arvensis*	7.4 ± 0.4 9.0 ± 0.8 6.1 ± 0.5 8.0± 0.1 7.4 ± 0.2 7.4 ± 0.1 8.0 ± 0.6	21.2 ± 0.3 28.3 ± 0.1 – 14.7 ± 0.4 15.7 ± 0.1 14.8 ± 0.5 15.4 ± 1.0	3 5 – – – – –	+ + – – – – –
Shigella spp. (SM-07)	*C. copticum* *C. sinensis* *C. copticum* *C. copticum*	13.2 ± 0.2 8.0 ± 0.4 8.3 ± 0.2 9.0 ± 0.8	*C. sinensis* *C. juncea* *S. aromaticum* *M. arvensis*	8 ± 0.4 – 19.5 ± 0.2 –	8.0 ± 0.4 – 19.5 ± 0.2 –	– – – –	– – – –
SM-08	*C. copticum* *C. copticum* *C. copticum* *C. copticum* *C. copticum*	9.0 ± 0.8 9.0 ± 0.8 9.0 ± 0.8 6.3 ± 0.3 6.5 ± 0.2	*C. sinensis* *C. juncea* *C. longa* *S. aromaticum* *M. arvensis*	6.3 ± 0.2 7.4 ± 0.4 – 8.0 ± 0.7 6.5 ± 0.2	15.6 ± 1.2 16.4 ± 0.9 – 14.3 ± 0.9 12.5 ± 0.1	– – – – –	– – – – –

Table 8 Synergistic interaction between Plant extract and antibiotics

Strains Used	Plant extract (P)	r_P (in mm)	Antibiotic (A)	r_A (in mm)	Combined radius ($r_P + r_A$) in mm	Enlargement of Zone-size (in mm)	Synergism
SA-03	*C. copticum*	6.0 ± 0.4	C	16.8 ± 0.7	25.1 ± 0.4	3	+
	C. copticum	6.0 ± 0.4	T	17.1 ± 0.8	23.4 ± 0.5	–	–
	C. copticum	6.0 ± 0.4	Gm	14.0 ± 0.7	24.5 ± 0.5	4	+
	C. copticum	6.0 ± 0.4	Am	14.0 ± 0.2	23.0 ± 0.6	3	+
	C. copticum	6.0 ± 0.4	NA	–	–	–	–
SA-08	*C. copticum*	7.0 ± 0.8	C	11.0 ± 5	23.1 ± 0.5	5	+
	C. copticum	7.0 ± 0.8	T	12.0 ± 0.5	19.1 ± 0.3	–	–
	C. copticum	7.0 ± 0.8	Gm	12.0 ± 0.5	22.1 ± 0.4	3	+
	C. copticum	7.0 ± 0.8	Am	10.1 ± 1.0	17.0 ± 0.5	–	–
	C. copticum	7.0 ± 0.8	Na	10.0 ± 0.3	17.8 ± 1.5	–	–
	C. copticum	7.0 ± 0.8	Cf	16.2 ± 0.6	23.8 ± 0.6	–	–
SM-07	*C. copticum*	5.5 ± 0.2	C	11.3± 0.17	16.3 ± 0.4	–	–
	C. copticum	5.5 ± 0.2	T	5.1± 0.1	10.0 ± 0.3	–	–
	C. copticum	5.5 ± 0.2	Am	8.4 ± 0.2	13.6 ± 0.3	–	–
	C. copticum	5.5 ± 0.2	Na	–	–	–	–
	C. copticum	5.5 ± 0.2	Cf	8.2 ± 0.5	13.4 ± 0.7	–	–
EC-20	*C. copticum*	8.3 ± 0.3	C	14.3± 0.2	22.6 ± 0.5	–	–
	C. copticum	8.3 ± 0.3	T	16.3 ± 0.1	24.4 ± 0.2	–	–
	C. copticum	8.3 ± 0.3	Gm	15.2 ± 0.3	27.3 ± 0.3	–	–
	C. copticum	8.3 ± 0.3	Am	12.1 ± 0.1	20.4 ± 0.2	–	–
	C. copticum	8.3 ± 0.3	Na	11.4 ± 0.1	19.6 ± 0.2	–	–
	C. copticum	8.3 ± 0.3	Cf	20.4 ± 0.1	28.4 ± 0.2		

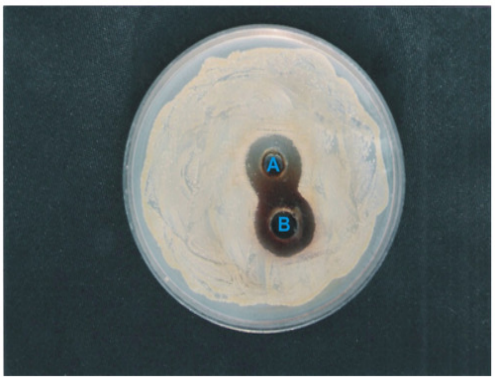

Plate 1 Synergistic interaction between plant extracts against *S. aureus* (SA-08)
(A) *P. guajava*
(B) *H. spicatum*

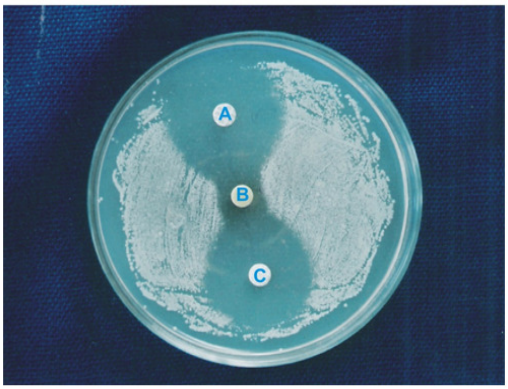

Plate 2 Synergistic interaction of plant extracts with antibiotics against *S. aureus* (SA-08)
(A) Gentamycin
(B) *C.copticum*
(C) Chloramphenicol

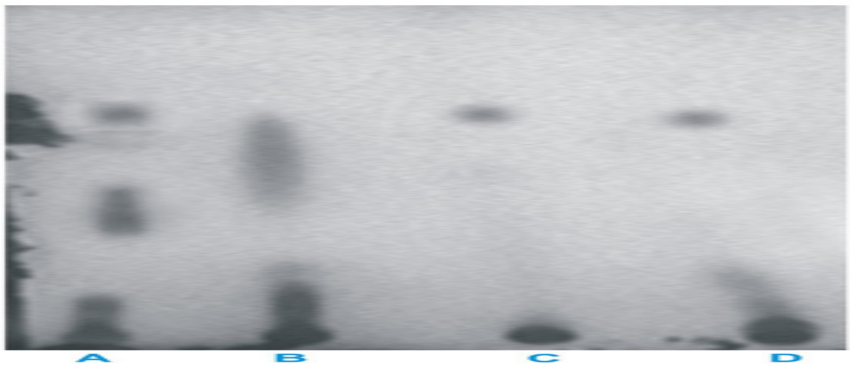

Figure 1 TLC profile for alkaloids of different plant extracts
Lane A: *A .barbadensis*; Lane B: *Z. officinale;* Lane C: *P. guajava and* Lane D: *H. spicatum*

Green Synthesis and Characterization of Zinc Oxide (ZnO) and Silver Nano particles (np) and Determination of Their Antibacterial Assay

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Abstract- Development of reliable and eco-friendly processes for synthesis of metallic nano particles is an important step in the field of application of nanotechnology. The synthesis of nano particles has become the matter of great interest in recent times due to its advantageous properties and applications in different fields. Though physical and chemical methods are more popular for nano particle synthesis, the biogenic production is a better option due to eco-friendliness. The present abstract reports the investigation, synthesis and characterization of Zinc Oxide (ZnO) and Silver nano particles (NP), and their application on pathogenic bacteria. ZnO NP were synthesized by chemical reduction method using starch as capping agent and silver NP was prepared by green synthesis process from $AgNO_3$ solution through the extract of *Citrus sinensis* (Santra). The detail characterization of the nano particles was carried out using UV-Vis Spectroscopy,X-Ray Diffraction (XRD) analysis, Scanning Electron Microscopy (SEM), and Thermogravimatric (TGA) analysis. From SEM image analysis, the average particle size was found to be 90 nm and 50 nm, for ZnO and silver nano particles respectively. From the analysis of XRD pattern, UV-VIS spectroscopy and TGA, the formation of nano particles was confirmed. Antibacterial assay of synthesized ZnO and silver NP was carried out both in liquid and solid growth medium against different pathogens.

Introduction

Green chemistry is a design, development, implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment. In the synthesis of metal nano particle by the reduction of the corresponding metal ion salt solutions. Nano particles are often referred to as clusters, nanospheres, nanorods and nanocups are just a few of the shapes at the small end of the size ranges from 1 to 100nm. Nano particles exhibit a number of special properties relative to bulk material and often have unique visible properties because they are small enough to confine their electrons and produce quantum effects [1]. Biosynthesis of nano particles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis [2]. A great deal of effort has been put into the biosynthesis of inorganic material, especially metal nano particle using microorganisms and plants [3-4]. The rate of reduction of metal ions using plants has been found to be much faster as compared to micro-organisms and stable formation of metal nano particles has been reported. The shape and size of the nano particles synthesized using plants can be controlled and modulated by changing the pH [5]. The reduction of silver ions (Ag+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers. Medicinal herbs are the local heritage with global importance. Medicinal herbs have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolite in one or more parts of these plants.

Citrus sinensis belongs to the family *Rutaceae* is an evergreen tree of Garhwal Himalaya includes about 17 species distributed throughout the tropical and temperate regions .Citrus peels contain more bioactive compounds, such as phenolic acids, flavonoids, limonoids, and fibre[6]. In traditional Chinese medicine, the dried peel of the fruit is used to treat abdominal distension, to enhance digestion, and to reduce phlegm, and its various parts are used to cure cutaneous complaints, hemiplegia, snake bite, fever, loss of taste, chronic rheumatism, stomach ache, menorrhagia, spleenomegaly, edema and cardiac diseases, bronchitis and asthma [7].Experimental studies have demonstrated its analgesic, antibacterial, antimicrobial, antiviral, antiyeast antifungal, antidiarrheal, antiinflammatory, uricosuric activity, antimutagenic, antisplasmodic, antiatherogenic, antiperoxidative activity, anticarcinogenic activity, and radical scavenging activity[8].

In the present investigation, synthesis and characterization of Zinc Oxide (ZnO) and Silver nano particles (NP), and their application on pathogenic bacteria were investigated. ZnO NP were synthesized by chemical reduction method using starch as capping agent and silver NP was prepared by green synthesis process from AgNO3 solution through the extract of *Citrus sinensis* (sweet lime).

Material and Methods

Chemicals

Pure and analytical grade chemicals were used in all experiments including synthesis of ZnO and silver nanoparticles, media preparation for growth of bacterial cells. Zinc nitrate hexa -hydrate, sodium hydroxide (NaOH) starch and silver nitrate ($AgNO_3$), were purchased from Himedia laboratories Pvt. Ltd., Mumbai, India. The bacterial cultures of *E.coli, Bacillus subtilis, Psudomonas aeruginosa, Streptococcus pneumonia* were obtained from Department of Microbiology SBSPGCBS Balawala Dehradun and Antibiotics, (Ampicilin, Tetracycline, Ciprofloxacin, and Tobramycin) were purchased from Himedia, Mumbai, India. Beef extract, peptone, sodium chloride (NaCl) were purchased from Merck, India.

Glassware and apparatus

All glass wares (Conical flasks, Measuring cylinders, Beakers, Petri plates and Test tubes etc.) were purchased from borosil, India.

Synthesis of ZnO nano particles

The ZnO nano particles were prepared by wet chemical method using zinc nitrate and sodium hydroxides precursors and soluble starch as stabilizing agent. Soluble starch (0.5%) was dissolved in 500 ml of distilled water and treated in microwave oven for complete solubilization. Zinc nitrate, 14.874 g (0.1 mol), was added in the above solution. Then the solution was kept under constant stirring at room temperature using magnetic stirrer for one hour. After complete dissolution of zinc nitrate, 300ml (0.2 mol), of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 hrs after complete addition of sodium hydroxide. After the completion of reaction, the solution was allowed to settle for overnight and the supernatant solution was then discarded carefully. The remaining solution was centrifuged (Remi cooling centrifuge instrument, Model No-C30BL) at 10,000 × g for 10 min and the supernatant was discarded. Thus produced nano particles were washed three times using distilled water. Washing was carried out

Synthesis of silver nano particles from fruit peels extract of Citrus sinensis

to remove the byproducts and the excessive starch that were bound with the nano particles. After washing, the nano particles were dried at 80°C for overnight. During drying, complete conversion of Zn (OH)$_2$ into ZnO takes place.

Ag nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon resonance. On mixing the extract with aqueous solution of the Ag ion complex a change in the color from colorless to yellowish brown was observed. It was due to the reduction of Ag+ which indicates the formation of Ag nanoparticles shown in Figure.

Extraction

Weighing 25 g peels of *Citrus Sinensis* was thoroughly washed in distilled water, dried, cut into fine pieces and was smashed into 100 ml sterile distilled water and filtered through Whatman No.1 filter paper two or three times. The extract was stored in refrigerator for further experiments.

Synthesis

The aqueous solution of 1mM silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Citrus sinensis* extract was added into 90 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions and kept for incubation period of 12 hrs at room temperature. Here the filtrate act as reducing and stabilizing agent for 1 mM of $AgNO_3$.

Characterization Techniques

UV-Vis Spectroscopy

The UV-Vis spectra of ZnO NP prepared with 0.5% concentration of soluble starch was shown in Figure (a). The absorption peak of the prepared nano ZnO was found at around 360 nm. The UV-Vis absorption spectra of the Ag NP were shown in Figure (b). Absorption spectra of Ag nano particles formed in the reaction media has absorbance maxima at 421 nm. A remarkable broadening of peak at around 350 nm to 480 nm indicates that the particles are polydispersed. It was observed that the peak was blue shifted in the absorption spectrum from 350nm to 480 nm with increasing reaction time.

The ZnO and Ag nano particles spectra obtained was followed the reported results (Vigneswaran et al., 2006 and Jain et al., 2009)

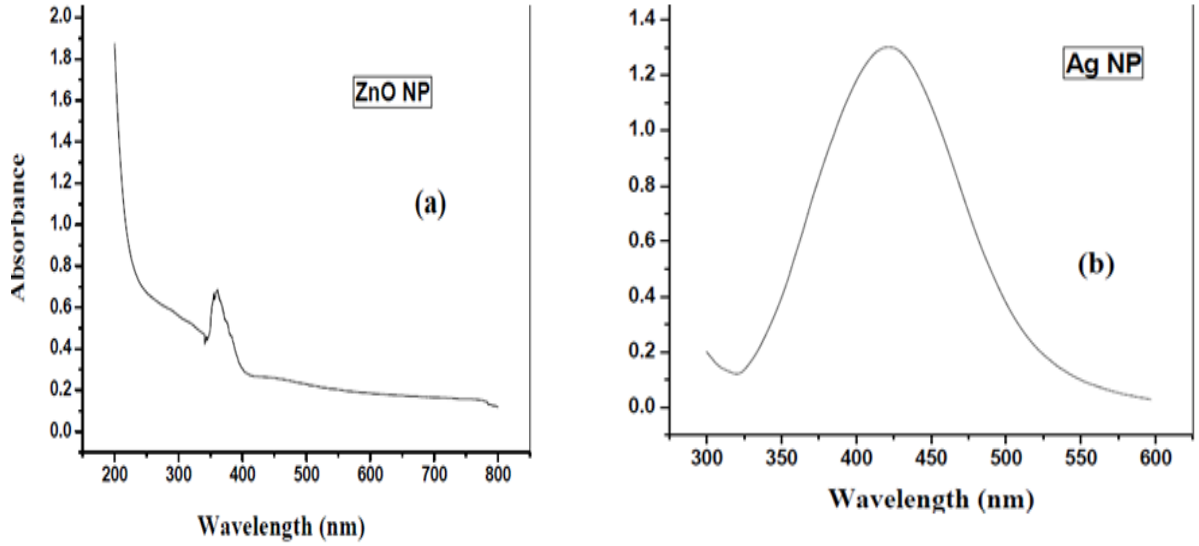
X-RAY diffraction method

The XRD pattern of bulk ZnO and nano ZnO were studied. All the peaks were hexagonal and approximately close to the reported information. Due to the crystal symmetry and related face velocities, the common crystal habit of ZnO is hexagonal in shape. Also the ZnO NP is the thermodynamically stable crystallographic phase. The width of the peaks in case of ZnO NP has increased due to the quantum size effect. The average particle size was estimated to be 42 nm using Scherer equation. The *Citrus sinensis* extract-mediated synthesized Ag nanostructure was confirmed by the characteristic peaks observed in the XRD image. All diffraction peaks correspond to the characteristic face centered cubic (FCC) silver lines. These diffraction lines observed at 2è angle 32.80, 38.20, 55.10° and

65.70 respectively, have been indexed as (111), (200), (220) and (311) respectively. XRD patterns were analyzed to determine peak intensity, position and width, full-width at half-maximum (FWHM) data was used with the Scherer formula. The typical XRD pattern revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver
nano particles. The average estimated particle size of this sample was 50 nm derived from the FWHM of peak corresponding to 111 plane with cubic and hexagonal shape.

Scanning electron microscope (SEM)

In this research work, Jeol JSM-6480 LV SEM machine were used to characterize mean particle size, morphology of nanoparticles. The ZnO powder sample and freeze dried sample of Ag NP solution was sonicated with distilled water, small drop of this sample was placed on glass slide allowed to dry. A thin layer of platinum was coated to make the samples conductive Jeol JSM-6480 LV SEM machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-20 kV. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached



(a)UV- Vis spectra of the ZnO with 0.5% of soluble starch

Table–1. Zone of Inhibition of Antibacterial test of ZnO NP

Bioactive agent		Zone of inhibition (Diameter, cm)			
		E.coli	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Streptococcus pneumonia*
ZnO nanoparticle	5 mM	3.2	2.2	nil	0.2
	10 mM	4.7	4.1	nil	3.1
	15 mM	4.8	4.8	3.1	4.9
Erythromycin(10mcg/disc)		0.8	0.7	0.6	4.8
Vancomycin(10mcg/disc)		nil	nil	nil	0.6
Tobramycin(10mcg/disc)		nil	3.1	nil	3.2

Table–2. Zone of Inhibition of Antibacterial Test of Ag NP

Bioactive agent		Zone of inhibition (Diameter, cm)			
		E.coli	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Streptococcus pneumonia*
Ag nanoparticle	0.2 mM	2.5	3.2	3.1	3.2
	0.3 mM	3.4	4.2	3.3	3.4
	0.6 mM	4.2	4.3	4.2	3.8
Erythromycin(10mcg/disc)		nil	nil	0.6	4.1
Vancomycin(10mcg/disc)		0.8	nil	0.8	3.8

with the SEM. The EDX analysis of ZnO and Ag sample was done by the SEM (JEOLJSM 5800) machine. The EDX normally reveals the presence of phases.

The SEM image of ZnO and Silver nanoparticles synthesized by chemical reduction method and green synthesis process by using 10 % fruit extract and 1mM AgNO3 concentration was very clear. It gave a clear image of highly dense ZnO and silver nanoparticles. The SEM image showing silver nano particles synthesized using *Citrus sinensis* extract confirmed the development of silver nanostructures.

Thermo gravimetric analysis (TGA)

Thermal decomposition behavior of the gel has been studied using Netzsch (STA 449C) DSC/TG. The DSC/TG patterns were collected as a function of temperature up to 9000C under N2 atmosphere. The heating rate was 100C/min. in N2. Alpha alumina was used as reference material. TGA or thermo gravimetric analysis ZnO NP was carried out to observe characterstic weight loss with temperature. The TGA analysis of synthesized nano ZnO was studied. The synthesized nano ZnO is subjected to a heating from room temperature to highly thermal temperature of 1000°C.The initial weight loss took place at around a temperature of 180°C.The bulk ZnO did not show any weight loss when heated up to 1000°C but in case of soluble starch a weight loss at around 312°C was observed, which is matching the results reported in (Vigneswaran et al., 2006) equal to the degradation temperature of starch. These properties helped to characterize and confirm the formation of ZnO nano particles (range obtained from 90-100nm).

Antibacterial test

Antibacterial assays

ZnO and Ag nano particles bactericidal effect was studied against four different pathogenic bacteria *E.coli, Bacillus subtilis*, *Pseudomonas aeruginosa, Streptococcus pneumonia*. These nano particles were dispersed in autoclaved Millipore water by ultrasonication. Aqueous dispersion of ZnO and Ag nano particles of desired concentration was made. Disc diffusion test of ZnO and Ag nano particles was also done.

Results and Discussion

The effect of different concentration of ZnO NP like-5mM, 10mM, 15mM and 0.2mM, 0.3mM and 0.6mM of silver nanoparticles on bacteria was performed. As we increased the concentration of ZnO nanoparticles the antibacterial activity of ZnO nanoparticles increased. A clear inhibition zone treated with ZnO nanoparticles whereas the standard antibiotics like vencomycin, erythromycin, and tobramycin shows smaller zone of inhibition as compared to the nanoparticles treated discs. A clear inhibition zone treated with Ag nanoparticles whereas the standard antibiotics like vencomycin, erythromycin shows smaller zone of inhibition as compared to the nanoparticles treated discs (Table-1 and 2).

References

1. Armendariz, V.; Gardea- Torresdey, J. L.; Herrera, I.; Jose-Yaceman, M.; Peralta-Videa, J.R. and Santigo, P. *Journal of Nanoparticles Research*, 2004; **6**(4), 377.
2. Bhattacharya, D. and Gupta, R.K. Nanotechnology and Potential of Microorganisms. Critical Reviews in Biotechnology, 2005; **24**(4), 199.
3. Mohanpuria, P.; Rana, N.K. and Yadav, S.K. Biosynthesis of nano particle: Technological concepts and Future applications. *Journal of Nano particles Research,* 2007; **7**, 9275.
4. Farooqui, M.A.; Chauhan, P.S.; Krishnamoorthy, P. and Shaik, J. *Digest Journal of Nano materials and Biostructures,* 2010; **5**(1), 43 .
5. Gardea-Torresedey, J.L.; Gombez, G.; Jose-Yaceman, M.; Parsons, J.G.; Peralta-Videa, J.R. and Tioani, H. *Journal of Hazardous Substance Research,* 2003; **4,** 1.
6. Du, Qizhen and Chen, Hui. The methoxyflavones in Citrus reticulata Blanco cv. ponkan and their antiproliferative activity against cancer cells Original Research Article, *Food Chemistry* 2010; 2:567-572
7. Li, Xian.; Sun, ChongDe.; Zhang, WangShu.; Zhou, ChunHua. and Chen, KunSong. Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species Original Research Article, *Food Chemistry* 2007; 4: 1338-1344.
8. Sacchetti, G.; Maietti, S.; Muzzoli, M.; Scaglianti, M.; Manfredini, S. and Radice, M. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry* 2005; 91: 621–632.

Phytochemical Screening and Antioxidant Activity of *Andrographis paniculata* (Burm. f.) Nees by DPPH Scavenging Assay

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Abstract- The aim of this present study is to explore the antioxidant capacity and phytochemical analysis of the whole plant extracts of *Andrographis paniculata.* The screening of phytochemical tests revealed the presence of some active ingredients such as alkaloids, tannins, saponins, phenols, terpenoids and flavonoids. The different solvent extract were also evaluated for their total phenolic contents and antioxidant capacity by Folin Ciocalteu reagent method and Free radical scavenging capacity (DPPH assay). Butylated hydroxy toluene was used for antioxidant capacity and gallic acid for phenolic content as a standard. The EC_{50} value of Acetone extract was 25.80 µg/ ml compared to 17.75µg/ml, for BHT. The result shows that acetone extract of the whole plant of *Andrographis paniculata* is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

Key words- *Andrographis paniculata,* DPPH, Phytochemicals, Phenolic compounds and Antioxidant capacity.

Introduction

Plants have been used as an alternative source of medicine from ancient times before the advent of synthetic drugs. Medicinal plants have played an important role in treatment of the world health. According to an approximation of World Health Organization, nearly 80% of the population of developing countries relies on traditional medicine. Therefore, such plants should be investigated for better understanding of their therapeutic properties, safety and efficacy[1,2]. The traditional folk medicinal system uses the plant-products for the treatment of various infectious diseases. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[3,4]. Several Indian medicinal plants are known for their beneficial therapeutic effects which also might have antioxidant properties[5]. Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA[6].

Andrographis paniculata(Burm. f.) Nees, one of the important medicinal plant, belongs to the family of Acanthaceae. It is plentifully found in south eastern Asia i.e., India, Sri Lanka, Pakistan and Indonesia. It is an annual herbaceous plant widely cultivated in India, China and some parts of Europe. It is found in wild throughout plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Orissa, Uttar Pradesh and Uttarakhand. Andrographis paniculata, "Kalmegh" of Ayurveda is an erect annual herb extremely bitter in taste in each and every part of the plant body. The plant is known in north-eastern India as 'Maha-tita', literally 'king of bitters'. Mostly leaves and roots have been traditionally used over centuries for different medicinal purposes in Asia and Europe as a folklore remedy for a wide spectrum of ailments or as a herbal supplement for health promotion.

The aim of the present study is the screening of the phytochemical constituents and antioxidant capacity of the extracts of Andrographis paniculata. Additionally, the inter-relationship between phenolic content and antioxidant capacity has been also carried out.

Material and Methods

Collection and authentification of plant

The plant sample *Andrographis paniculata* (Burm. f.) Nees was collected from Forest Research Institute, Dehradun. The authentification of plant sample has been certified by taxonomist. The whole plant was

dried in the shade and made into coarse powder using an electrical grinder. The powdered sample was preserved in airtight bags till further use.

Solvent extraction of plant material

The powdered material (100gms) was extracted for 24 hrs successively in Petroleum Ether, Chloroform, Acetone and Methanol by using Soxhlet apparatus. The extracts were concentrated in vacuum evaporator and kept in vacuum desiccators for complete removal of solvent and the weight of each extract was measured.

Preliminary phytochemical screening

The various solvent extracts of the plant samples were then subjected to qualitative chemical tests for the identification of various phytochemical constituents like Alkaloids, Carbohydrates, Glycosides, Proteins, Tannins, Sterols, Saponins, Amino acids etc ,which are responsible for pharmacological as well as biological activity of plant.

Detection of alkaloids

Small portion of the solvent free extract was stirred with a few drops of diluted HCl and was filtered. The filtrate may be tested for following color tests Mayer's reagent (cream colored precipitate); Hager's reagent (yellow colored precipitate and Wagner's reagent (reddish brown precipitate) to detect the presence of an alkaloid.

a) Mayer's test: Test solution with Mayer's reagent (1.36 g of mercuric chloride in 60 ml distilled water + 5.0 g of potassium iodide in 20 ml distilled water + 20 ml of distilled water) gave cream precipitate.

b) Hager's test: Test solution with Hager's reagent (saturated aqueous solution of picric acid i.e. 1.0% (v/v) solution of picric acid in hot water) gave yellow precipitate.

c) Wagner's test: Test solution with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 5 ml of water and 100 ml distilled water) gave reddish brown precipitate.

Detection of carbohydrates and glycosides

Small quantity of extracts were dissolved separately in 4 ml of distilled water and filtered. The filtrate may be subjected to Molish's Test; Selivanoff test; Barfoed's test; Fehling's test and Benedict's test to detect the presence of carbohydrate qualitatively.

a) Molish test: Test solution with few drops of Molish's reagent (10 gm napthol) in 100 ml of 95% of alcohol and 2 ml of conc. H2S04 added slowly from the side of test tubes showed a purple ring at the junction of the two liquids.

b) Selivanoff's test: An appearance of pink colour on addition of resorcinol crystals and conc. HCI in test solution, detected the presence of carbohydrates.

c) Fehling's test: Test solution neutralized by addition of sodium hydroxide solution and treated with Fehling A and B (added in equal volume) gave precipitate.

d) Barfoed's test: Test solution treated with Barfeod's reagent (12 gm of copper acetate in 200 ml distilled water and addition of 12.5 ml of 8.5 % of lactic acid solution) when boiled on water bath gave brick red precipitate.

e) Benedict's test: Test solution treated with Benedict's reagent and allowed to boil on a water bath, showed reddish brown precipitate.

Detection of sterols

Sterols were detected by performing following tests.

a) Salkowaski's test: When a few drops of conc. H_2So_4 were added to the test solution, shaken and allowed to stand, lower layer turned red, indicating the presence of sterols.

b) Liebermann – Burchard's test: The test solution was treated with few drops of acetic anhydride. When concentrated sulphuric acid is added from the sides of the test tube, it showed a brown ring at the junction of the two layers and upper layer turned green.

c) Hensen's test: Portion of dried extract were taken in 10ml of chloroform. To this few drops of conc. H_2SO_4 was added. Both the acid and chloroform layers turning red which shows the presence of sterols.

Detection of saponins

Dilute 1 ml of the test solution with distilled water to 20 ml and shake in a graduated cylinder for 15 mins. A 1 cm layer of foam indicated the presence of saponins.

Detection of phenolic and flavonoid Compounds

Small quantity of tests dissolved in water and subjected for following tests to detect the presence of phenolic compounds and tannins.

a) $FeCl_3$ solution (5%) test: Test solution treated with a few drops of ferric chloride solution, the presence of intense green colour indicates presence of phenolic compounds.

b) Vanillin HCl acid test: Test solution was treated with

vanillin reagent (19ml Vanillin in 10 ml alcohol and 10 ml concentrate HCl), presence of red color indicate presence of phenolic compounds.

c) Zinc hydrochloric acid reduction test: Test solution with zinc dust and few drops hydrochloric acid, shows magenta red color.

Detection of proteins and free amino acids

Dissolved small quantities of test solution in a few ml of water and subjected the solution to following test:

a) Millon's Test: Test solution when treated with Millions reagents (1 gm mercury in 9 ml fuming HNo_3, volume maked upto 100ml with distilled water) and heated on a water presence of red precipitate, indicates presence of amino acids.

b) Ninhydrin test: Test solution treated with Ninhydrin reagent (0.1 % *w/v* of ninhydrinin an n-butanol) presence of blue violet colour indicates presence of amino acids.

c) Biuret test: Test solution treated with 40% NaOH and dil copper sulphate solution, presence of blue colour indicates presence of amino acids.

Test for tannins

Portion of extracts were taken separately in a few ml of alcohol. To this few drops of freshly prepared FeCl3 (1.62gm in 1L of .001M HCl) was added. Development of greenish violet color shows the presence of tannins.

Free radical scavenging capacity (DPPH assay)

The hydrogen donating ability of each extract in the presence of DPPH (2, 2-diphenyl-1-picrylhydrazyl) stable radical was examined according to method developed by Blois[7] and Cao *et al*[8]. The stock solution of DPPH was prepared and was kept at 20°C. 2ml of a volume of 25 to 200 µl of the sample was then poured into an optical glass cuvette and the stock solution was added to a final volume of 4ml. After 30 min incubation at 28 to 30°C in dark, the absorbance at 517 nm using spectrophotometer (Thermofisher Scintific UV-2700) was measured against a blank of pure methanol and DPPH (2ml each). Synthetic antioxidant Butylated Hydroxy Toluene (BHT) was used as a standard. The experiment was repeated thrice. The antioxidant capacity was expressed as Ec50 value, which represents the sample concentration necessary to decrease the initial DPPH concentration by 50%.

The Ec50 value was calculated as follows-

$$\%\ DPPH\ radical - scavenging = \left[\frac{Absorance\ of\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control} X\ 100\right]$$

Ec50 of the extract was determined by using SPSS software.

Determination of the total phenolic content

Total phenolic contents were determined by Folin Ciocalteu Reagent Method[9] using gallic acid as a standard. Appropriate dilutions of the samples (0.25ml) were oxidized with Folin-Ciocalteau reagent for five minutes at room temperature followed by addition of 7.5% (w/v) Sodium carbonate solution. The absorbance of the resulting blue color was measured at 740nm on UV-VIS spectrophotometer after heating at 50°C for 5.0 minutes in a water bath. Quantification was done on the basis of the standard curves of gallic acid prepared from 1ml of each of the 200,150,100 and 50 ppm gallic acid solutions assaying in the manner similar to that used for the extracts. The experiment was repeated thrice and the results were expressed in term of % gallic acid equivalents (GAE).

Results and Discussion

The result of the screening of phytochemical constituents of *Andrographis paniculata* is summarized in Table – 1.0

From the Table-1 it is clear that *Andrographis paniculata* possesses variety of phyto- chemicals. Among all the solvent extracts, the acetone and methanol extracts are rich in phyto chemical constituents. So these two solvent extract were further examined for antioxidant activity. The antioxidant capacity was determined using DPPH free radical scavenging assay. DPPH is a stable free radical which accepts electrons and becomes a stable diamagnetic molecule. The purple color or violet color of the DPPH solution changes to yellow and the absorbance at 517nm wavelength maximum decreases. The decrease in absorbance of DPPH caused by antioxidant activity is due to the reaction between the antioxidant molecule and its radical which results in the scavenging of the radical by electron donation. Acetone extract shows good antioxidant activity; so the results of acetone extract was only shown. Table-1.2 reveals that the acetone extract had DPPH radical scavenging activity which ranges from 51% to 90% at a concentration of 17ìg/ml. In acetone extract possess scavenging more than 90% compared to the BHT control. The BHT control was assayed at 100 ìg/ ml corresponding to the maximum allowable concentration for BHT additions to food stuffs[10]. The EC50 value for

Table-1.0 Phytochemical constituents of *Andrographis paniculata*

S. No.	Phytochemical Test	Petroleum ether extract	Chloroform extract	Acetone extract	Methanol extract
1.	**Test for carbohydrates**				
i	Fehling's test	(-)	(-)	(-)	(+)
ii	Molish's test	(-)	(-)	(-)	(-)
iii	Barfoed's test	(-)	(-)	(-)	(-)
iv	Benedict's test	(-)	(-)	(-)	(+)
v	Selivinoff's test	(-)	(-)	(+)	(+)
2.	**Test for proteins and amino acid**				
i	Million's test	(-)	(-)	(+)	(-)
ii	Biuret test	(-)	(-)	(+)	(-)
iii	Ninhydrin test	(-)	(-)	(+)	(-)
3.	**Test for steroids**				
i	Salkowski test	(+)	(+)	(+)	(+)
ii	Gilberman-Buchard's test	(+)	(+)	(-)	(-)
iii	Hensen's test	(-)	(-)	(+)	(+)
4.	**Test for alkaloids**				
i	Wagner's test	(-)	(-)	(+)	(+)
ii	Hager's test	(-)	(+)	(+)	(+)
iii	Mayer's test	(-)	(-)	(-)	(-)
5.	**Test for phenolic and flavonoid compounds**				
i	Vanillin-HCl test	(-)	(-)	(+)	(+)
ii	Ferric chloride test	(+)	(+)	(-)	(+)
iii	Zinc hydrochloric acid reduction test	(-)	(-)	(-)	(-)
6.	**Test for tannins**	(-)	(-)	(-)	(+)
7.	**Test for saponins**	(-)	(-)	(+)	(+)

(+)present, (-) absent

Table-1.1 Free Radical Scavenging Capacity of Acetone Extract

S. No.	Concentration of Acetone extracts (µg/ml)	Antioxidant activity (%)
1	25	54.50
2	50	61.84
3	75	66.81
4	100	76.65
5	125	83.16
6	150	75.16
7	175	80.41
8	200	85.41
9	Ec_{50}	25.80

Table-1.2 Results of Total Phenolic Content in Acetone Extract

Solvent Used	Total Phenol content (%)	Ec_{50} (%)
Acetone	0.213	25.80

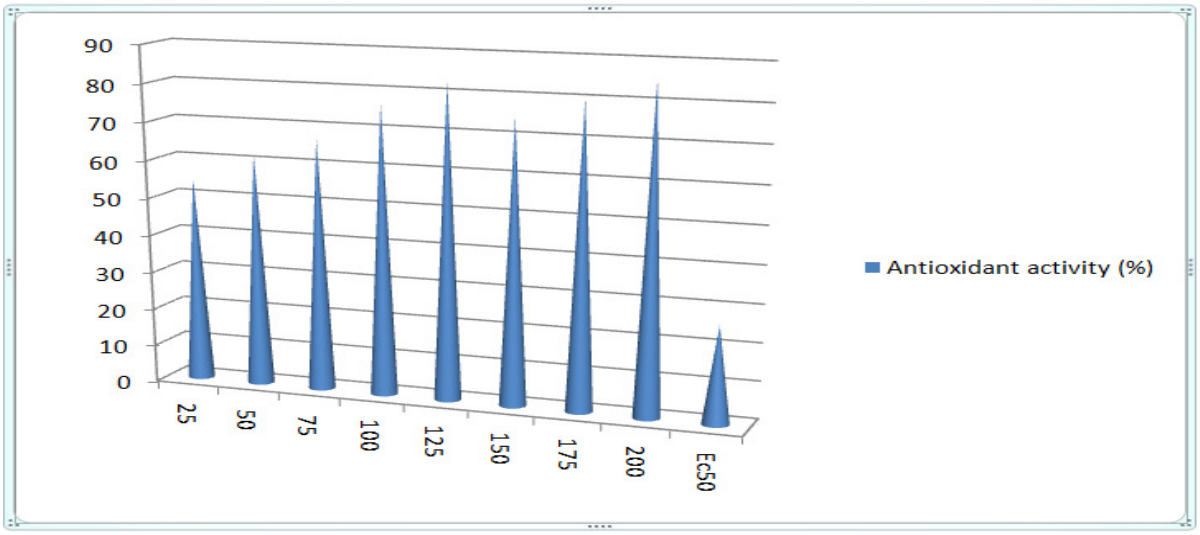

Fig. Antioxidant Activity (%) of Acetone extract at different concentration

acetone was found to be 25.80 ìg/ ml, which is higher than that of the standard value. DPPH free radical scavenging capacities of the plant extractive are attributed to their phenolic constituents. A large number of studies have been reported[11, 12] wherein the free radical scavenging capacity of the plant positively correlated with their phenolic constituents. Thus the acetone extracts was also examined for its phenolic constituents. Total phenolic constituents is determined by Folin Ciocalteu reagent method For the acetone extract the value comes around 0.24% Gallic Acid Equivalents (GAE). It was observed that the phenolic contents of acetone extracts are associated with an increased radical scavenging capacity. This effect may be due to the electron donating ability of the phenolic compounds present in the acetone extract.

Conclusion

The above data confirms that the plant *Andrographis paniculata* is rich in phyto-chemical constituents. Further study explores the in-vitro antioxidant capacity of acetone extracts with results comparable to those of the standard compounds such as Butylated Hydroxy Toluene (BHT), gallic acid. *Andrographis paniculata* can therefore be proposed as a new potential sources of natural additives for the pharmaceutical industries. The data clearly indicated that the acetone extracts of *Andrographis paniculata* possessed good antioxidant activity among all three solvent extract. However, further work needs to be conducted to isolate the components responsible for the antioxidant activities of the extracts and to identify bioactive compounds.

References

1. Iuliano, L.; Luigi, I.; Colavita, A.R.; Leo, R.; Pratico, D. and Violi, F. Oxygen free radicals and platelet activation. *Free Radical Biology & Medicine*, 1997; 22: 999-1006.
2. Nascimento, G.G.F.; Lacatelli, J.; Freitas, P.C. and Silva, G.L.; GL, " Antibacterial activity of plants extracts and phytochemicals on antibantibiotic-resistance bacteria", *Braz. J. Microbiology*, 2000; Vol. 3, No: 4 pp. 886-891.
3. Halliwell, B.1995. How to characterize an antioxidant: an update. Biochem.Soc.Symp., 61: 73-101.
4. Osawa, T. 1994. Novel natural antioxidants for utilization in food and biological systems. In Uritani, I.; Garcia, V.V. and Mendeoza, E.M. (Eds.). Postharvest biochemistry of plant food-materials

in the tropics (pp. 241-251). Tokyo, Japan: Japan Scientific Societies Press.
5. Amudha P. and Shanthi P., antioxidant activity of some rare medicinal plants *Journal of Pharmacy Research*, 2011; 4(3), 698-699.
6. Iuliano, L.; Luigi, I.; Colavita, A.R.; Leo, R.; Pratico, D. and Violi, F. Oxygen free radicals and platelet activation. *Free Radical Biology & Medicine*, 1997; 22, 999-1006.
7. Blois, M.S. and Zhao, X.Y. Antioxidant determinations by the use of a stable free radical, *Nature*, 1958; 181, 1199-1200.
8. Cao, G.; Sofic, E. and Prior, R. L. Antioxidant and Pro-oxidant Behavior of Flavonoids:
9. Structure Activity Relationships, *Free Radical Biol. Med*., 1997; 22, 749-760.
10. Singleton, V.L. and Joseph, A.R. Jr. Colorimetry of Total Phenolics with Phosphomolybdic- Phosphotungstic Acid Reagents, *Am. J. Enol. Vitic*, 1965; 16:3, 144-158.
11. Ribeiro, S.M.R., Barbwa, L.C.A.; Qweiro, J.H.; Knodler, M. and Schieber, A. Phenolic compounds and antioxidant capacity of Brazilian Mango (*Mangifera indica* L.), *Food chem*., 2008; 110, 620-626.
12. Yang, J.H.; Lin, H.C. and Mau, J.L.; Antioxidant properties of several commercial mushrooms, *Food Chem.*, 2002; 77, 229-235.
13. Kaur, C. & Kapoor, H.C. Antioxidant activity and phenolic content of some Asian vegetables, *International Journal of Food Science and Technology*, 2002; 37, 153-161. ●

Comparative Phytochemical Screening and *in-vitro* Free Radical Scavenging Activity and Anti-microbial Activity of Leaves and Rhizomes of *Acorus calamus* Linn.

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Abstract- The present communication attempts to evaluate the comparative *in-vitro* activity of leaves and rhizomes of *Acorus calamus* Linn. (Araceae family). *Acorus calamus* Linn.is a well known medicinal plant in traditional medical systems having various ethanopharmacological uses. As the official source of plant was roots and rhizomes and it had been studied extensively. Previously leaves of *Acorus calamus* were not regarded as useful part of plant, but now-a-days there is growing interest in leaves of this plant as there is no detailed work reported so far on its leaves. The free radical scavenging activity of methanolic extract of leaves and rhizomes of the plant was investigated *in-vitro*, using spectroscopic method against 1, 1-diphenyl-2- picrylhydrazyl (DPPH). The leaf extract was found to possess strong activity against DPPH as compared to rhizome. Antimicrobial activity was also performed using ethanolic extract through cold percolation method against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherischia coli and Salmonella typhi.* The extract was found to have positive results against all.

Keywords: *Acorus Calamus* Linn., ethanopharmacological, free radical scavenging, DPPH, Antimicrobial.

Introduction

Acorus calamus Linn. is semi-aquatic herb with creeping rhizomes and sword shaped long leaves, found near marshy places, river banks and lake.[1] It is up to 6 feet tall, aromatic, sword shaped leaves bearing small yellow/green flowers and branched rhizome. It is widely distributed throughout India and Ceylon, in marshes, wild or cultivated, ascending the Himalayas up to 6000 feet in Sikkim, marshy tracts of Kashmir and Sirmoor in Manipur and Naga Hills.[2] The roots and rhizomes are used medicinally since ancient times. They possess antispasmodic, carminative and anthelmintic properties and are also used for the treatment of epilepsy, mental ailments, chronic diarrhoea, dysentery, bronchial catarrh, fever and glandular and abdominal tumours.[3,4] They are also employed for kidney and liver troubles, rheumatism, sinusitis, eczema and anti-cellular activities.[5] Recently roots and rhizomes identified as antibacterial agent against fish pathogen[6] and also shows insulin sensitizing activity.[7] Whereas mature green leaves exhibit various activities including insect repellent, when cut up and stored with dry foods[8], antihyperlipidemic activity, antidiabetic activity[9], antipsychotic activity[10], antimicrobial and analgesic actions.[11] As we all know that it is increasingly being realized that majority of the diseases today are due to the shift in the balance of pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free redicals or due to the excessive oxidation stress of the current life or the poor scavenging / quenching in the body due to the depletion of dietary antioxidants. Keeping in view of the above observations, in the present study, methanolic extract of leaf and rhizome of the plant were subjected to in-vitro antioxidant activity against the DPPH generated free radicals.

Material and Methods

The leaves were wildly collected from catchment of Bhimtal Lake in Uttarakhand located in North India, proclaimed as to have ethano-pharmacological importance. These were preserved in 70% ethyl alcohol for various studies.

Screening for free radical scavenging activity

Chemicals- 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Rutin, methanol, Standard DPPH solution: 0.135 mM solution, Sample stock solution: 0.1 mg/ml solution for all sample methanolic extract (1 mg/10 ml methanol).

Methodology- The effect of extract on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1 ml of extract in methanol containing 0.02-0.1

mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of mixture was measured spectrophotometrically at 517 nm; ascorbic acid, rutin were used as references. The ability to scavange DPPH radicals was calculated by this equation.

$$\text{DPPH radical scavenging activity (\%)} = \frac{(Abs_{control} - Abs_{sample})}{(Abs_{control})} \times 100$$

where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract / reference.

Screening for antibacterial activities

Chemicals- 10% aqueous dimethylsulfoxide (DMSO), Gentamicin (4µg/ml),

Zone of inhibition (Diffusion Method)- The dried extract was dissolved in 10% aqueous dimethylsulfoxide (DMSO)to a final concentration of 500mg/ml and sterilized by filtration through a 0.45µm membrane filter. Antibacterial activity was determined by agar well diffusion method. Bacteria were cultured at 30°C for 24 hrs in Muller Hinton Broth (MHB,Hi-media). An inoculum consisting of 10^6CFU/ml was used. Antibiotics such as Gentamicin (4µg/ml) and solvent 10% DMSO without the test compound were used as positive and negative controls respectively. The tests were conducted in triplicate.

Dtermination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the methol extract was evaluated by Tube dilution method . The methanol extract MIC was determined by dilution of the extract to various concenterations (15.625-500mg/ml). All the tubes were incubated to suitable temperature for 18-24hrs. The tubes were observed for any growth . The MIC was interpreted as the lowest concentration of the extract that did not show any visible growth when compared with control tubes.

Results and Discussions

Results obtained from the present study show that the leaf and rhizomes of *Acorus calamus* Linn. contain alkaloids, saponins, terpenoids, flavonoids, resins, essential oil, carbohydrate and tannin,. Results are reported in Table-1 and 2. Phytochemical screening of successive fractions from Soxhlet: (+) shows presence, and (“) shows absence of content.

Table- 1 Phytochemical screening of leaf of *Acorus calamus* Linn.

S. No.	Constituents	Tests	Hexane	Chloroform	Ethylacetate	Methanol	Water
1.	Carbohydrates	Benedict's test	+	+	+	+	+
		Molisch's test	+	+	+	+	+
		Caramelisation	+	+	+	+	+
2.	Glycosides	Fehling's test	-	+	+	+	+
3.	Steroids	Libermann burchard test	+	+	+	-	-
		Salkowski reaction	+	+	-	-	-
4.	Proteins & Amino acids	Biuret test	-	-	-	+	+
		Ninhydrin test	-	-	-	-	+
5.	Saponins	Foam test	-	-	+	+	+
6.	Tannins	$FeCl_3$ test	+	+	+	+	+
		Alkaline reagent test	-	-	+	+	+
		Vanillin hydrochloride test	-	-	-	+	+
7.	Triterpenoids	Libermann burchard test	+	+	+	-	-
8.	Alkaloids	Dragendroff's test	-	-	-	-	-
		Mayer's test	-	-	-	-	-
9.	Resin	Resin	-	-	-	-	-
10.	Flavonoids	Alkaline reagent test	-	+	+	+	+
		Shinoda's test	-	-	+	+	+

Table-2 Phytochemical screening of Rhizome of *Acorus calamus* Linn.

S. No.	Constituents	Tests	Hexane	Chloroform	Ethylacetate	Methanol	Water
1.	**Carbohydrates**	Benedict's test	-	-	+	+	+
		Molisch's test	-	+	+	+	+
		Caramelisation	-	+	+	+	+
2.	**Glycosides**	Fehling's test	-	+	+	+	+
3.	**Steroids**	Libermann burchard test	+	+	+	-	-
		Salkowski reaction	-	+	-	-	-
4.	**Proteins & Amino acids**	Biuret test	-	-	-	+	+
		Ninhydrin test	-	-	-	-	+
5.	**Saponins**	Foam test	-	-	+	+	+
6.	**Tannins**	$FeCl_3$ test	+	+	+	+	+
		Alkaline reagent test	-	-	+	+	+
		Vanillin hydrochloride test	-	-	-	+	+
7.	**Triterpenoids**	Libermann burchard test	+	+	+	-	-
8.	**Alkaloids**	Dragendroff's test	-	-	-	-	-
		Mayer's test	-	-	-	-	-
9.	**Resin**	Resin	-	-	+	-	-
10.	**Flavonoids**	Alkaline reagent test	-	-	-	-	-
		Shinoda's test	-	-	-	-	-

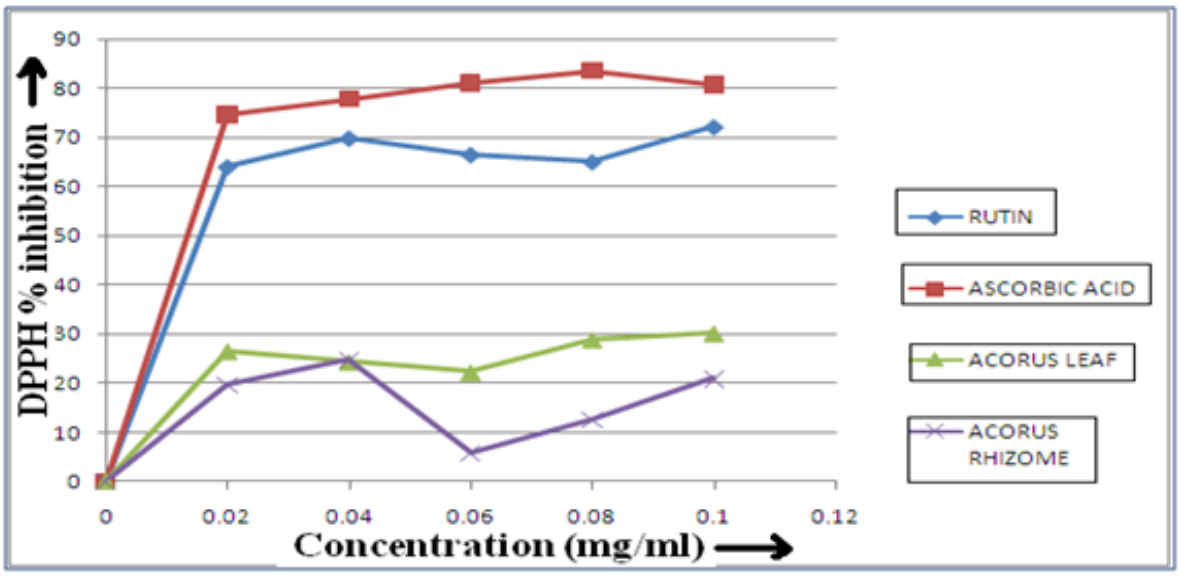
Table- 3 Absorbance at various concentrations

Concentrations →	0.02	0.04	0.06	0.08	0.1
Ascorbic acid	0.153	0.133	0.114	0.099	0.116
Rutin	0.217	0.182	0.202	0.211	0.168
Leaf	0.443	0.450	0.469	0.429	0.421
Rhizome	0.485	0.454	0.569	0.528	0.477

Table- 4 Percentage of Standards and Sample

Concentration	0.02	0.04	0.06	0.08	0.1
Ascorbic acid	74.62%	77.94%	81.09%	83.58%	80.76%
Rutin	64.01%	69.81%	66.5%	68.01%	72.13%
Leaf	26.53%	24.37%	22.22%	28.85%	30.18%
Rhizome	19.56%	24.70%	5.63%	12.43%	20.89%

Fig.1 DPPH % Free radical inhibition Vs Concentration mg/ml.



Free radical scavenging activity- In-vitro DPPH free radical scavenging activity of the methanolic extract of the leaf and rhizome of *Acorus calamus* Linn. Compared with Ascorbic acid and Rutin (standard used) it was observed that extract of the leaves shows higher activity than that of the rhizome. At a concentration of 0.1 mg/ml the scavenging activity of the leaves reached 30.18% while at the same concentration rhizome have 20.89% activity. Absorbance of control was 0.603. Results are shown in table- 3, 4 and Fig. 1

Antimicrobial activity (cup plate method)

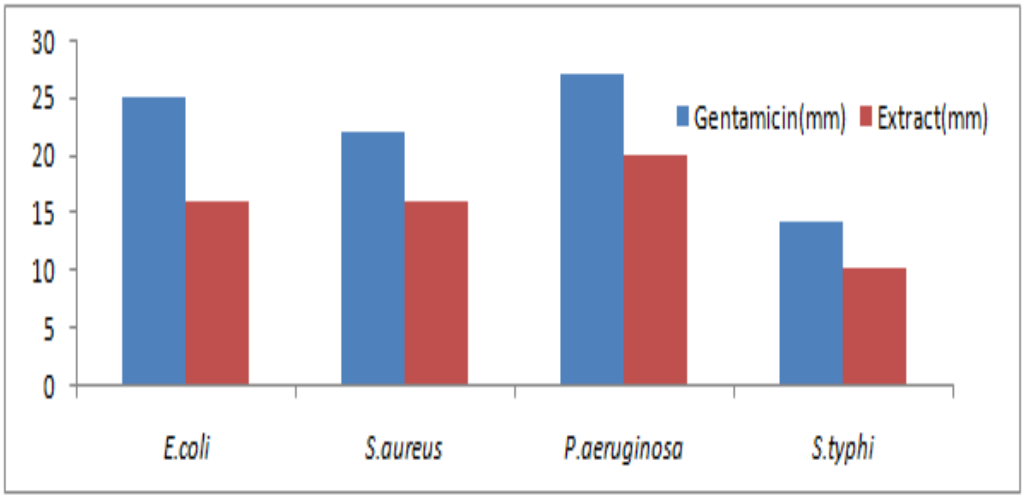
Table- 5 Zone of inhibition (mm)

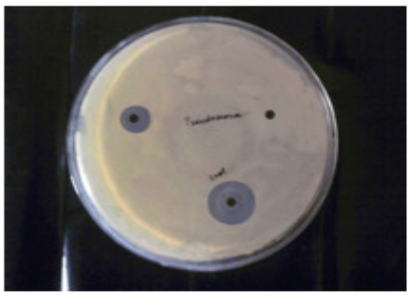
	E.coli	*S.aureus*	*P.aeruginosa*	*S.typhi*
Gentamicin	25	22	27	14
Extract	16	16	20	10

Table- 6 Minimum Inhibitory Concentration (mg/ml) of Extract

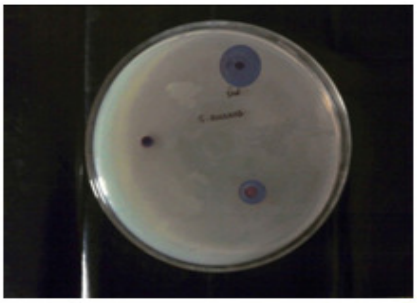
E.coli	*S.aurues*	*P.aeruginosa*	*S.typhi*
125	125	62.5	250

Fig.2 Comparative graph between Gentamicin and EtOH extract showing zone of inhibition (mm)

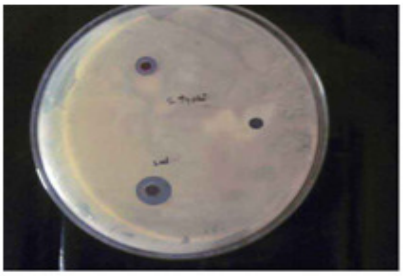




P.aeruginosa (std= gentamicin, B=blank, E=Extract)



S.aureus (std=gentamicin, B=blank, E=Extract)



S.typhi (std= gentamicin, B=blank, E=Extract)

E.coli (std= gentamicin, B=blank, E=Extract)

Fig.3 Plates showing zone of inhibition against various micro-organisms.

Acknowledgment

Authors are thankful to the Head Mr. Yogesh Joshi and Management, Himalayan Institute of Pharmacy and Research, Rajawala, Dehradun for providing facilities and encouragement.

Conclusion

Results of the proposed study suggest that there is possible use of leaf and rhizomes of *Acorus calamus* Linn. as a natural antioxidant. As it is already known that free radicals have implicated in causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, CNS disorders etc. Study of Acorus *calamus* Linn leaf and rhizome shows considerable antioxidant activity. Thus, it may be concluded that the plant can be effectively used further in the treatment of above mention ailments. It may also be concluded that the present property of the plant is due to the presence of high level of polyphenolic compounds including flavonoids, flavonols, proanthocyanidines etc. Plant also shows positive and considerable anti-microbial activity against all micro-organisms used.

References

1. Rastogi, R.P. and Mehrotra, B.N. *Acorus calamus.Compendium of Indian medicinal plants.*1980; Vol 3, 11.
2. Rao, M.M.; Meena, A.K.; Singh, A. and Kumari, S. Physicochemical and preliminary phytochemical studies on the rhizomes of *Acorus calamus* Linn, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2010; 2 (2). 130-31.
3. Chopra, R.N.; Nayar, S.L. and Chopra, I.C. *Glossary of Indian medicinal plants,* 2002; NISCAIR, CSIR, Delhi.

4. Nadkarni, K.M. *Indian Materia Medica*, 3rd Edn., Vol. I, Popular Prakashan Pvt. Ltd. Bombay, 1982; 1211-12.
5. Agarwal, S.L.; Dandia, P.C.; Singh, K.P. and Arora, R.B. A note on the preliminary studies of certain pharmacological actions of *Acorus Calamus* L.,*J. Am. Pharm. Assoc*., 1956; 45. 655-56.
6. Bhuvaneswari, R. and Chellam, B. Anti bacterial activity of *Acorus calamus* and some of its derivates against fish pathogen *Aeromonas hydrophila*, *Journal of Medicinal Plants Research*, 2009; 3(7). 538 47.
7. Hao-Shu Wu; Di-Feng Zhu; Chang-Xin Zhou; Chu-Rui Feng; Yi-Jia Lou; Bo Yang and Qiao-Jun He. Insulin sensitizing activity of ethyl acetate fraction of *Acorus calamus* L. *in-vitro* and *in-vivo*, *Journal of Ethnopharmacology*, 2009; 123 (2). 288-92.
8. Chan Park; Soon-Il Kim and Young-Joon Ahn. Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored-product insects., *Journal of Stored Products Research*, 2003; 39. 333–42.
9. Reshma, S. and Sushma, A. Hypolipidemic activity of *Acorus calamus* L.in rats., *Fitoterapia*, 2002; 73 (6). 451-55.
10. Pandy, V.; Jose, N. and Subhash, N. CNS activity of methanol and acetone extracts of *Acorus calamus* leaves in mice, *J. Pharmacol. Toxicol*., 2009; 4. 79-86.
11. Devi, A.S. and Ganjewala, D. Antimicrobial activity of *Acrous calamus* L. rhizomes and leaf extract, *Acta Biologica Szegediensis*, 2009; 53 (1) 45-49.
12. VengadeshPrabu, K.; George, T.; Vinoth, K.R.; Nancy, J.; Kalaivani, M. and Vijayapandi, P. Neuromodulatory effect of *Acorus calamus* leaves extract on dopaminergic system in mice. *International Journal of PharmTech Research*, 2009; 1(4) 1255-59.
13. Hyeri Kim; Tae-Ho Han and Seong-Gene Lee**.,** Anti-inflammatory activity of a water extract of *Acorus calamus* L. leaves on keratinocyte HaCaT cells, *Journal of Ethnopharmacology*, 2009; 122 (1). 149-56.
14. World Health Organization: *Quality control methods for medicinal plant materials.* Published by WHO, Geneva, 1998.

Synthesis of Zno Nano Particles Using Plant Extract, Their Characterization and Antibacterial Activity

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Abstract - Zinc is a trace element and essential nutrient in humans. It plays a vital role in all physiological processes. The aim of the present study is to synthesize ZnO nano particles by sol-gel process using zinc acetate dihydrate as the starting material and ethanolic extract of sunflower seeds as surfactant. The prepared sample was then calcined at two different temperatures 300° and 600 °C. The synthesized samples were characterized using XRD, FE-SEM and EDAX to determine their morphology, composition, particle size and structure. The results obtained show agglomeration up to certain extent and the particle size is close to ~30 nm for calcination at 600 °C.

Antibacterial activity was performed using the MIC test for *E.Coli* (gram-negative) bacteria. Optical density of the samples was measured after the 14 hour incubation period and compared to the positive controller to determine the efficiency. Both the samples gave almost similar results probably due to high degree of agglomeration. The calcination temperature when increased, particles tend to obtain a specific shape such as acicular or rod like. In case of antibacterial testing, a refined shape gives better results if the particle size is similar. If not, then the efficiency of the process will go down as the particle size increases, shape remaining the same.

Keywords: ZnO, sunflower seeds extracts, *E.Coli* bacteria, Antibacterial activity

Introduction

Zinc is an essential nutrient for human health. It is a natural element found in all plants and animals and plays a crucial role for the growth of skin, teeth, bones, hair, nails, muscles, nerves and brain function. In other words, Zinc is a trace element and plays a vital role in all physiological processes in humans. Its function in cells and tissues depends on those metallo-proteins and enzymes with which it is associated[1]. It has been used as a drug in the prevention and treatments of diseases since 14th century A.D.[2], as a number of zinc-containing formulations including Rasaka or kharpara (zinc ore or zinc carbonate), Yasada (zinc metal), pushpanjana (zinc oxide) and pittala (brass) are used as therapeutic agents in Ayuveda[3]. Therapeutic uses of zinc oxide in modern system of medicine have also been used. Zinc oxide is widely used in cosmetics as it shows antibacterial action along with UV protection effect[4, 5].

It has been well established that nano materials have different properties as compared to their bulk counterpart, therefore, use of ZnO nano particles as an antibacterial agent is widely investigated these days[6, 7]. ZnO nano particles are also considered to be non-toxic, bio-safe and biocompatible. The antibacterial activity of ZnO has been studied largely with different pathogenic and non-pathogenic bacteria such as *S. aureus and E. coli*.[6, 7]. Several reports have addressed the harmful impact of nano materials on living cells, but relatively low concentrations of ZnO are nontoxic to eukaryotic cells 8-11. ZnO nano particles significantly inhibit growth of a wide range of pathogenic bacteria under normal visible lighting conditions[7]. Several studies suggest that different morphologies (particle size and shape) of ZnO have different degrees of antibacterial activities[7, 12]. The most accepted mechanism of the antibacterial activity of ZnO is that it develops Reactive Oxygen Species (ROS) including hydrogen peroxide, hydroxyl ions and singlet oxygen, which migrate into the bacterial cell and eventually disrupts the membrane[7]. Other reasons cited for the same are electrostatic forces in ZnO nano particles that directly kill bacteria[13] and the surface abrasiveness of ZnO nano particles which was reported to produce disorganization of both cell wall and cell membrane of *E. Coli*[12].

In the present paper, synthesis of ZnO nano-particles has been carried out by sol-gel method using alcoholic (ethanol) extract of sunflower seeds (oleic acid as one of the constituent[14]) as surfactant and potassium hydroxide as precipitating agent and were then calcined. The synthesized and calcined samples were characterized by different characterizing techniques XRD, FE-SEM, EDAX. Antibacterial testing of the prepared samples using *E. coli* bacteria were evaluated.

Experiment

Synthesis of natural surfactant

Dried powder of sunflower seeds were taken in a round bottom flask to this ethanol was added in 1: 5 w/v ratio. The mixture was refluxed at 60 °C for 24 h. The solution thus obtained was cooled to room temp. and then filtered to remove the residue. The volume of etanolic solution was reduced up to half the volume by rotary evaporator and was used as surfactant.

Synthesis of ZnO nano-particles

In the present study sol-gel method is used to prepare ZnO nano-particles[15]. Aqueous solution of zinc acetate dihydrate (50 ml, 0.2M) and DMSO (50 ml) were taken in a beaker and stirred magnetically. To the resulting solution 5 ml of ethanolic extract of sunflower seeds[14] was added as surfactants and the stirring was continued for half an hour. To this, 1.2M potassium hydroxide solution was added drop-wise to initiate the precipitation reaction and stirring was continued for another two hours. Centrifugation of the sample was done to obtain the precipitate. The precipitate thus obtained was washed with water repeatedly to remove the impurities of K^+. It was then washed with ethanol to remove the impurity of organic solvents. The precipitate obtained after washing was dried at 60-70 °C.

Characterization

Thermal analysis of the milled ZnO powder was carried out using TG/DTA (Perkin-Elmer Diamond) in air at a heating rate of 10 °C/ min to determine the calcination temperature. The structure of synthesized ZnO powder was studied by the Powder X-Ray Diffractometer (XRD, D8 Bruker AXS Diffractometer) with CuKá radiation (ã= 0.1541 nm) and a monochrometer 50 kV and 300 mA with the scanning rate and step being 2 °/min and 0.02°, respectively. The surface morphology of the powder and coatings was examined by a Field Emission Scanning Electron Microscopy (FESEM, Quanta FEI-200) and a Scanning Electron Microscope (SEM, ZEISS EVO-18). Qualitative elemental analysis of the coatings was carried out using an Energy Dispersive Analysis of X-rays (EDAX, PENTA FET Precision) an attachment with the FESEM equipment.

Antibacterial testing of the prepared samples

ZnO nano particles as prepared were subjected to a quantitative antibacterial testing procedure. MIC test was employed and Optical Density (OD) values were measured at 600 nm. To determine the antibacterial activity an inoculum of *E.Coli* bacteria (gram negative) was prepared using LB broth and nutrient agar as a medium. All the test tubes were then put in autoclave for killing of any residual impurities. The samples were measured for 5 different weights including 0.5, 0.75, 1, 1.5 and 2 mg. Samples were then put in the bacteria medium and kept for incubation for 14 hours. Optical density of the tubes was then measured at 600 nm for various samples. A positive control was kept in which no ZnO particles were added. Optical Density value of the samples was then compared with the positive control and efficiency was calculated.

Results and Discussion

TGA results

Thermal Gravimetric Analysis (TGA) was carried out for the sample as prepared. In TGA, weight loss of a substance is measured under controlled heating rate as a function of temperature. This was done to determine suitable temperatures for calcination. Weight loss is observed when the temperature is suitable for the reaction. Hence in a particular temperature range (25-1000 °C) with heating rate (10 °/min) and in air atmosphere (with gas flow 200 ml/min), a gradual decrease in weight is observed from the thermogram shown in Fig. 1.

The weight loss observed in the temperature range 22-141 °C is not considered for calcinations as this temperature range is associated with weight loss due to moisture and melting of some foreign substances. From the TG curve, it has been observed that a signifi-

cant weight loss is observed in the temperature range 400 °C to 555 °C. This indicates that some structural or phase change takes place within this temperature range. Hence, calcination temperatures were chosen as 300 °C and 600 °C so that the effective phase change may take place for comparison.

XRD analysis

XRD of the calcined samples was carried out to confirm the presence of ZnO compound. It also indicated the impurities if any, present in the samples. XRD spectra are shown for the samples calcined at 300 °C (Fig. 2) and at 600 °C (Fig. 3).

In the XRD spectra of the powder obtained after calcination at 300° and 600 °C, it has been observed that the compounds of only ZnO (JCPDS card No. 00-036-1451) and ZnO_2 are present with minimum noise distortion or impurities. However, for the sample calcined at 600 °C sharp peaks of ZnO indicate the presence of particles in highly crystallized format (JCPDS card No. 01-080-0074). In the spectra mostly the peaks obtained are of ZnO. Whereas, $K_2Zn_6O_7$ peaks are also observed indicating that complete washing of potassium ions from the samples did not take place.

FESEM analysis

FE-SEM of the calcined samples was done to determine their morphology, particle size, extent of agglomeration, etc. The HV was kept at 20kV and images up to a magnification of 100,000X were obtained as shown in Fig. 4.

The average particle size obtained for the sample calcined at 300 °C is around 20-30 nm. A lot of agglomeration is evident from the figure, which indicates that the amount of surfactant used for the present study is not sufficient to prevent the agglomeration effectively. However, it has been observed from the figure that the shape of the particles is mostly spherical and globular. For the sample calcined at 600 °C, no apparent shape or morphological change has taken place in comparison to the sample calcined at 300 °C. Particle size around 20-30 nm, is almost similar to the sample calcined at 300 °C.

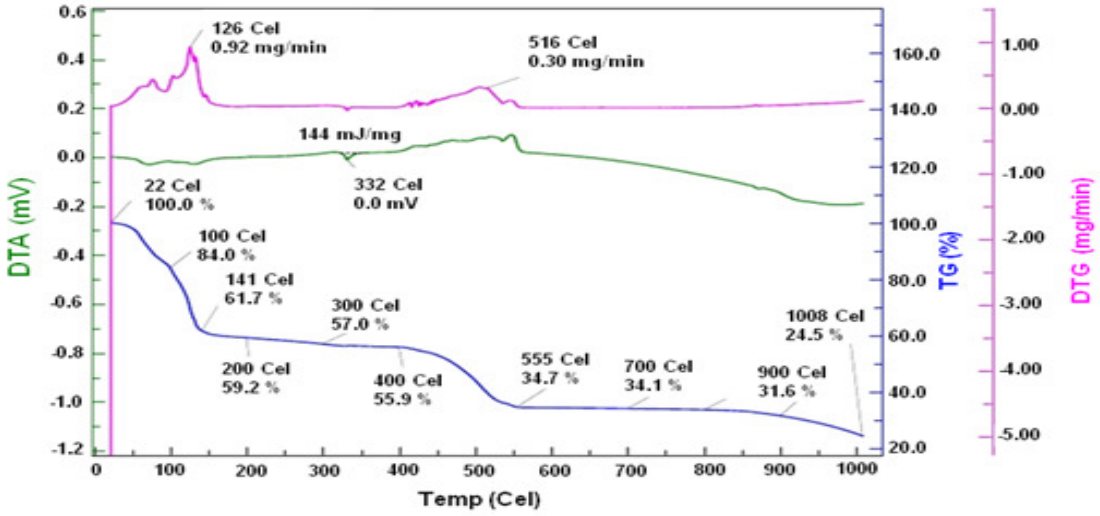


Fig. 1 Thermal Gravimetric Analysis curve of as-synthesized sample.

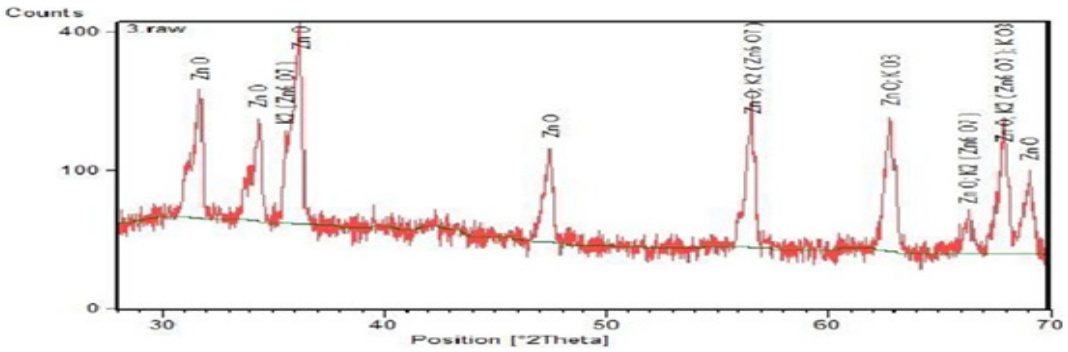


Fig. 2: XRD spectrum of synthesized sample using alcoholic extract of sunflower seed as surfactant and calcined at 300 °C.

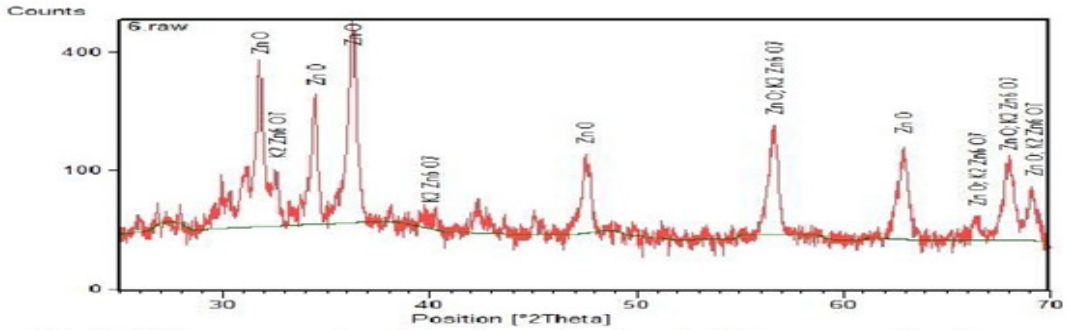
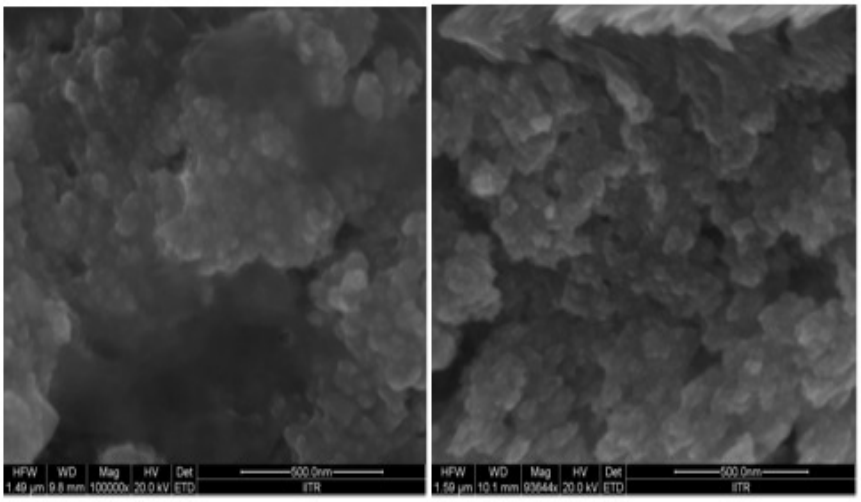


Fig. 3: XRD spectrum of synthesized sample using alcoholic extract of sunflower seed as surfactant and calcined at 600 °C.



(a) (b)

Fig. 4 FESEM images of synthesized nano particles obtained on calcination at (a) 300 and (b) 600 °C.

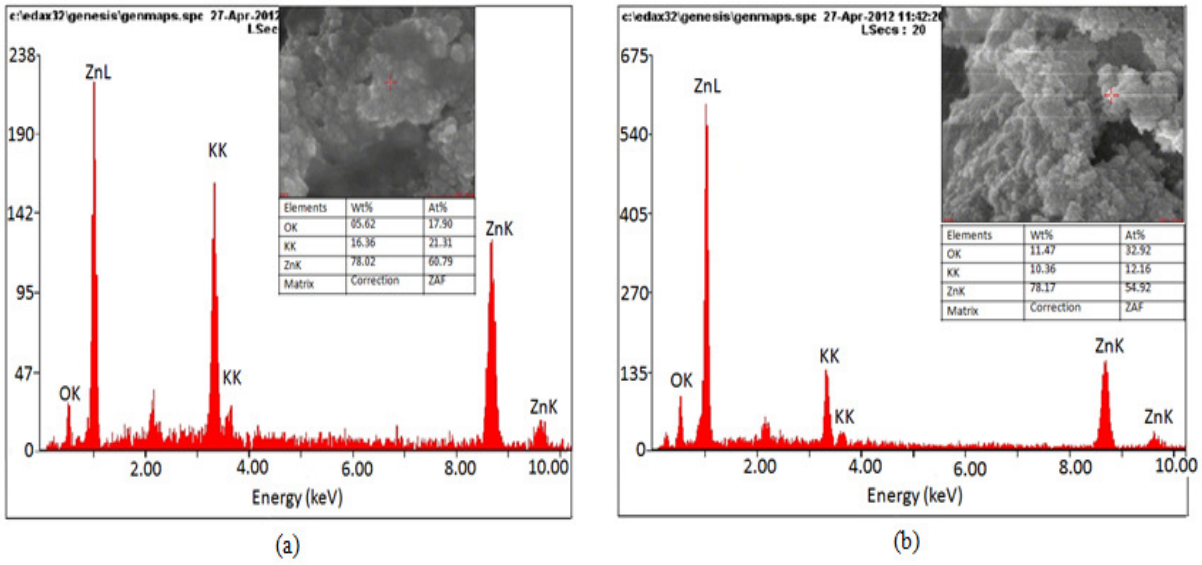


Fig. 5: EDAX micrograph of the sample calcined at (a) 300 °C and (b) 600 °C.

EDAX analysis

Energy dispersive X-ray spectroscopy (EDS) is an analytical technique used for the elemental analysis or chemical characterization of a sample. The prepared samples were characterized using EDAX technique (Fig. 5) to determine the elemental composition. Mostly peaks from the Zn K and O K shells were obtained. Zn L peaks were obtained in the graph; however, it was not incorporated in the table.

During synthesis, KOH was used as precipitating agent. After synthesis, repeated washing was done to remove K^+ ions, but from the XRD analysis, it has been observed that K^+ ions were not completely removed even with repeated washing. This is also clearly evident from the elemental composition table (see Fig 5), though, the major element is still Zn with sharp and well-defined peaks of K and L shell. Since point imaging was done to obtain the EDAX analysis results *i.e.*, on varying the location report of elemental analysis may vary. This might be the reason for a deviation from the elemental composition obtained for sample calcined at 300 °C in comparison to sample calcined at 600 °C. Amount of K^+ ions vary in this case that means K^+ is present in free form and particles formed are mostly of ZnO. The atomic percent of Zn as obtained from its K- shell area is 60.79.

Antibacterial activity test

The samples prepared were tested for their antibacterial properties against *E.Coli.* (gram negative) bacteria. Optical Density (OD) values were measured after an incubation period of 14 hours and compared to the positive controller to determine the efficiency of the process. Each sample was tested for 5 different concentrations such as 1.5, 1.875, 2.5, 3.75 and 5 mM. Following equation was used to determine the efficiency of the sample and the data obtained is given in the Table- 1.

$$\frac{\text{ODE value positive controller - ODE sample}}{\text{ODE value positive controller}} \times 100 = \text{Efficiency}$$

Sample (calcinations temp.)	Weight (mg) for 5ml	Concentration (mM)	Optical Density value	Efficiency %
Positive control	0	-	1.723	-
300	0.5	1.25	1.137	34.02
300	0.75	1.875	0.964	44.06
300	1.0	2.5	0.896	48.01
300	1.5	3.75	0.671	61.06
300	2.0	5	0.548	68.20
600	0.5	1.25	1.062	38.38
600	0.75	1.875	0.907	47.37
600	1.0	2.5	0.876	49.17
600	1.5	3.75	0.713	39.18
600	2.0	5	0.593	65.59

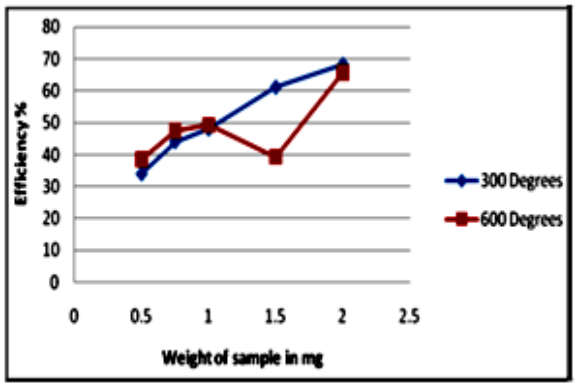


Fig. 6: Efficiency curve for 300 and 600 °C.

Efficiency of samples calcined at 300 °C and 600 °C were compared and given in Fig. 6. It can be observed that efficiency for both the samples is almost similar with results slightly better for 600 C sample. This might be due to the highly crystalline structure of the sample as suggested by XRD. Slight dip in the efficiency for the sample calcined at 600 °C might be due to inhomogeneity of the suspension or sample contamination. The efficiency of both the samples is in the range of 30-70%. This % efficiency may be explained in terms of degree of agglomeration. Particles were not separated properly which accounts to lower their effectiveness. Sample at 600 °C was slightly less agglomerated, hence its efficiency is better.

Conclusions

The following conclusion has been drawn from this study that Sol-Gel route is a cost effective route for synthesis of ZnO nano particles. However, the amount of surfactant/ capping agent is not sufficient to prevent the agglomeration of the sample but it effectively arrests the growth of nano particles (*i.e.*, 20-30 nm in size) as determined by FESEM. Calcination temperature has a pronounced effect on the morphology of particles but in the present case, only partial development of the morphologies took place which suggests that a higher temperature or greater calcinations time is needed to complete the transformation. For antibacterial applications, in the present case two forces are acting together (i) Particle size – the lesser the particle size, the greater is the efficiency against bacteria (ii) Extent of agglomeration has an inverse relation with bacterial efficiency. As when the particles are separated they will be better available for attack. The use of natural surfactant shows slightly better antibacterial activity to that of commercially available chemicals (ethylene diamine and citric acid monohydrate) used as surfactants reported in our previous paper[16] where antibacterial tests were carried out by zone of inhibition method.

Acknowledgement

The author Dr. Sulaxna is thankful to Uttarakhand State Biotechnology Department (USBD), Haldwani, for financial assistance.

References

1. Ray, P.C., History of Hindu Chemistry, Bharti Prakasana, Kolkata, 1902, Vol.1, pp 10-13.
2. Sarangadhara, Samhita, Purbardha, 3rd Chapter, verse no. 34-46, Chaukhamba Sanskrite Academy, Varanasi, 1st edn, 1998.
3. Dash, V. B., Alchemy and metallic medicine in Ayuurveda, Concept Publishing, Company, New Delhi, 1986.
4. Padmavathy, N. and Vijayaraghavan, R., Enhanced bioactivity of ZnO nanoparticles—*an antimicrobial study, Sci. Technol. Adv. Mater*., 2008; 9. 035004-11.
5. Applerot, G.; Perkas, Nina; Amirian, Galina; Girshevitz, Olga and Gedanken, Aharon "Coating of glass with ZnO via ultrasonic irradiation and a study of its antibacterial properties", *Applied Surface Science,* 2009; 256S. S3–S8.
6. Brayner, R.; Ferrari-Iliou, R.; Brivois, N. and Benedetti, S. Djediat, Toxicological Impact Studies Based on Escherichia coli Bacteria in Ultrafine ZnO Nanoparticles Colloidal Medium, Nano Lett. 6 (2006) 866–870.
7. Ray, N. B. Jones; Koodali, R.T. and Manna, A.C. Antibacterial activity of ZnO nano particle suspensions on a broad spectrum of micro organisms, FEMS Microbiol Lett., 2008; 279. 71–76.
8. Thill, A.; Zeyons, O.; Spalla, O.; Chauvat, F.; Rose, J.; Auffan, M. and Flank, A.M. Cytotoxicity of CeO_2 Nanoparticles for Escherichia coli. Physico-Chemical Insight of the Cytotoxicity Mechanism *Environ. Sci. Technol*., 2006; 40. 6151–6156.
9. Sawai, J.; Doi, R.; Maekawa, Y.; Yoshikawa, T. and Kojima, H. Indirect conductimetric assay of antibacterial activities, *Journal of Industrial Microbiology and Biotechnology,* 2002; 29. 296-298.
10. Sunada, K.; Kikuchi, Y. and Hashimoto, Y. Fujishima, Bactericidal and Detoxification Effects of TiO_2 Thin Film Photocatalysts, *Environ. Sci. Technol*., 1998; 32. 726–728.
11. Sunada, K.; Watanabe, T. and Hashimoto, K. J. Studies on photokilling of bacteria on TiO_2 thin film, *Photochem. Photobiol*., 2003; A 156. 227–233
12. Padmavathy, N. and Vijayaraghavan, R. Enhanced bioactivity of ZnO nano particles—an antimicrobial study, *Sci. Technol. Adv. Mater*., 2008; 9, Article No. 035004.
13. Stoimenov, P.K.; Klinger, R.L.; Marchin, G.L. and Klabunde, K.J. Langmuir, Metal Oxide Nano particles as Bactericidal Agents, 2002; 18 6679–6686.
14. Mohammed, A.S.; Lai, O.M.; Muhammad, S.K.S.; Long, K. and Ghazali, H.M., 'Moringa oleifera, Potentially a New Source of Oleic Acid-type Oil for Malaysia', Investing in Innovation, 2003; 137-140.
15. Singh, A.K.; Viswanath, V. and Janu, V.C. Synthesis, effect of capping agents, structural, optical and Photoluminescence properties of ZnO nanoparticles, *Journal of Luminescence,* 2009; 129. 874–878.
16. Sharma, S.; Naik, D. and Agarwala, V. Synthesis, Characterization and Antibacterial Activity of Synthesized ZnO Nano particles of Different Morphology, *Advanced Materials Research*, 2012; Vol. 585. pp 154-15.